Kelt Reconditioning and Reproductive Success Evaluation Research
Project Number 2007-401-00
Report covers work performed under BPA contract # 73354 REL 62 and 73354 REL 79
Report was completed under BPA contract #73354 REL 79
Report covers work performed from: January 2022 – December 2022

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Report Created:

“This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.”

This report should be cited as follows:

Hatch, D., R. Branstetter, J. Stephenson, A. Pierce, N. Graham, J. Newell, W. Bosch, S. Everett, P. Burrows, K. Scott, L. Medeiros, L. Jenkins, C. Ray, D. Cervantes, T. Cavileer, J. Nagler, L. Caldwell, M. Fiander, C. Frederickson, J. Blodgett, and R. Johnson. Kelt Reconditioning and Reproductive Success Evaluation Research. 1/1/2022 - 12/31/2022 Bonneville Power Administration Annual Report, 2007-401-00.

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Abstract

The Kelt Reconditioning and Reproductive Success Evaluation Project is a research, monitoring, and evaluation (RM&E) uncertainties category project, that was funded through the 2008 Columbia Basin Fish Accords. The objectives are to evaluate methodologies to produce viable artificially reconditioned repeat steelhead (*Oncorhynchus mykiss*) spawners and evaluate reproductive success, physiology, homing, and geographic differences. Our work occurs in both the Yakima and Snake river-basins, additionally we make some contrasts and comparisons with the Kelt Reconditioning Project in the Upper Columbia River (Project 2008-458-00). We focused on collecting steelhead kelts at juvenile bypass facilities at Prosser, Lower Granite, Little Goose, and Lower Monumental dams. These kelts were reconditioned (given prophylactic treatments and fed a specially formulated diet) at Prosser and Dworshak National Fish (DNFH) hatcheries. Survival of long-term reconditioned kelts has been 45% (23 years) at Prosser Hatchery and 39% (12 years; 43% over the last 10 years) for mixed stock collections reconditioned at both Nez Perce Tribal and Dworshak National Fish hatcheries combined. In total, we released 804 and 5,154 reconditioned kelt steelhead in the Snake and Yakima rivers since 2011 and 2000, respectively. In 2022, unmarked upstream "wild" migrant adult steelhead return counts to Bonneville Dam have improved, but are still on the low end for unclipped fish, which have been recorded since 1994. This places great importance on safety net programs such as the kelt reconditioning program to bolster stocks during low return years. Years with low runs typically translate into a lower abundance of kelts, but the value of each successfully reconditioned fish increases. In 2022, we collected 183 kelts in the Snake River which were taken to DNFH for reconditioning. There were 22 reconditioned-mature (a combination of consecutive and skip spawners) fish that were released into the Snake River below Lower Granite Dam on November 21, 2022. In 2022, we collected 119 kelts in the Yakima River. From this collection 74 reconditioned-kelts were released, of which 49 were determined to be mature consecutive spawners to the Yakima River below Prosser Dam on October 28, 2021. Reproductive success of reconditioned steelhead was confirmed in the Yakima River tributaries of Satus and Toppenish creeks, with genetic parentage assignments (4,649 samples from Satus and 4,194 samples from Toppenish collected and genotyped from 2013 through 2021). Lifetime reproductive success for female reconditioned kelt steelhead in the Yakima River is estimated as 2.67 relative to steelhead that successfully spawn once. Studies applying tools from fish physiology and endocrinology to issues in kelt reconditioning were ongoing in 2022. These studies aim to achieve a sufficiently detailed understanding of the physiology of reconditioning in kelt steelhead to provide a scientific basis for maximizing the success of reconditioning programs. Screening of kelts for maturation status using plasma estradiol levels has become an essential part of the project. In 2022, we sampled blood at DNFH, Winthrop and Prosser hatcheries, and provided maturation status of individual fish at DNFH and Winthrop to project managers so that consecutive and skip spawners could be managed appropriately. All fish regardless of maturation status were released at Prosser in 2022. The 2022 results were added to a comparison of the performance of the three Columbia River Basin kelt projects in terms of survival and maturation rates. In addition, we are continuing data analysis in a study where we are combining the results of a genetic analysis enabling classification of Yakima River kelts by subpopulation, with survival, physiological, and migration data. From 2008 to 2022, we have detected conclusive evidence of 161 kelts showing repeat homing and upstream movement patterns and an additional 1,360 kelts that is consistent with repeat homing in the Yakima basin. An RFP for construction of the Snake River Kelt Facility was released by BPA in the Spring of 2022. A single contractor responded to the RFP and the review team agreed to postpone the project. We expect to again release an RFP for construction of the facility in early 2023. The project is anticipated completion date is fall of 2024. Due to the ongoing COVID-19 pandemic restrictions which started to abate in 2022, the shutdowns of many conferences and travel were still in effect which has resulted in diminished opportunities for presentations. A comprehensive list of 2022 and prior presentations are available in the appendices along with peer-reviewed articles regarding steelhead kelts produced by CRITFC and its partners along with other accredited sources.

Acknowledgments

We thank our partners at the Yakama Nation, Upper Columbia Kelt Reconditioning Program (#2008-458-00), Tom Scribner, Keely Murdoch, Matt Abrahamse, Michelle Steg-Geltner, and John Box for sharing data and sampling with our team. Many thanks to the Lower Granite Dam capture and sampling crews (Nez Perce Tribe, and Army Corps of Engineers Walla Walla District) and the US Fish and Wildlife Service and Nez Perce Tribal Hatchery staff at Dworshak National Fish Hatchery. Thank you, Lori Maxwell at Hagerman Genetic lab, for diligent and reliable laboratory processing and collection of genotypic data for this project. Also, we appreciate the help from Tim Resseguie and his team at Yakama Nation Fisheries for helping us with electroshocking and genetic collection. We thank our colleagues at CRITFC: Jeff Fryer, Denise Kelsey, David Graves, Joe Nowinski, Jayson FiveCrows, Agnes Strong, Jon Matthews, Loretta Islas, Chris Roe, Sue Wallace, Lisa Wood, Jenny Thrasher, Christine Golightly, Julie Carter, Lauren Burns, and Amanda Kanter. Thanks to our long-time collaborators at the Yakama-Nation Klickitat Fisheries Program (Joe Blodgett, Justin Olney, JJ, OJ, and Anneliese Myers). We would like to also thank Washington Dept. of Fish and Wildlife, Tim Webster and his field crew.

Executive Introduction

Steelhead *Oncorhynchus mykiss* returns in the Columbia River have declined over the last two decades and counts of wild steelhead at Bonneville Dam haven't exceeded 50,000 since 2016. All steelhead stocks upstream of Bonneville are listed under the Endangered Species Act (NMFS 1996) which has been recently reaffirmed in the last NMFS review (NMFS 2022a & b). Segregated hatcheries, mark selective fisheries, and other management strategies have failed to recover wild steelhead stocks. We need to develop new approaches aimed at restoring wild steelhead stocks. Kelt steelhead are relatively abundant in the Columbia River even in the upper most reaches, however repeat spawners represent a very small proportion of the upstream run. Developing strategies to convert the abundance of kelt steelhead into increased numbers of repeat spawners is the primary goal of the project.

This effort was initiated to explore the notion of revitalizing post-spawn wild steelhead, returning them to the river, and adding production to the population. In strong collaboration with the Yakama Nation, Nez Perce Tribe, and University of Idaho we gained knowledge of fish collection, care, and culture, measured contributions of reconditioned kelt steelhead to wild production, developed an understanding of kelt physiology, measured reproductive success, homing, and revealed life history pathways of kelt steelhead. Since 2008, this kelt reconditioning project successfully reconditioned and released 5,627 repeat spawning steelhead in the Yakima and the Snake River basins. We documented our findings in 18 published manuscripts and 94 professional presentations since 2013. The project has matured from a concept to nearly ready for production scale operations in both the Yakima and Snake rivers. Future research should focus on analysis of skip spawner management strategies but other efforts such as measuring reproductive success, physiology discovery, and homing should sunset after production facilities come online.

Kelt reconditioning as a conservation tool is intended to enhance populations that have suffered decline. A near-term improvement in productivity can be a means to offset long and short-term demographic perils and minimize loss of genetic and life history diversity. Kelt reconditioning programs can work in conjunction with restoration or remediation efforts. Re-establishment or enhancement of repeat spawning in listed steelhead populations can improve productivity, diversity, and demographic stability and is particularly important during times of low steelhead abundance. The kelt project was acknowledged by National Marine Fisheries Service (NMFS 2019) as the only hatchery action in the basin that is directly improving wild steelhead abundance. In 2022, NMFS (2022a) reaffirmed that our program was beneficial to Snake River B-index populations.

The primary goals of the project include developing and testing strategies to increase the survival and abundance of natural-origin repeat spawning steelhead in target populations while minimizing potential deleterious effects. Our proposed work is to continue addressing questions first identified by the ISRP in a 2014 review of a sister project (Steelhead Kelt Reconditioning 2008-458-00) and used in this project's last ISRP review in 2022 (ISRP 2022). These questions include: Focus on identifying at-risk populations for collection utilizing genetic tools to focus reconditioning efforts on these populations specifically. Transferring kelt reconditioning knowledge and techniques to other at-risk populations in the Columbia River Basin to further assist in recovery efforts. Development of a plan to provide the documented kelt technological knowledge to other specific agencies for implementation.

Introduction, Problem Statement, Relevance, Goals and Objectives, and Study Area

Columbia Basin steelhead *Oncorhynchus mykiss* have experienced a dramatic decline in abundance, resulting in listing of all distinct population segments as threatened or endangered since the late 1990's (Busby et al. 1996). The NMFS in its recent reviews of both Mid-Columbia and Snake River steelhead populations have maintained their ESA status as threatened (NMFS a & b). A subset of the species of the family Salmonidae, including steelhead, exhibit iteroparity – the ability to spawn on two or more occasions after a period of recovery. Unfortunately, many of the factors believed to contribute to the decline of steelhead (NRC 1996; US v. Oregon 1997; ISRP 1999; NMFS 2022c) have also placed significant limitations on the opportunity for successful iteroparity. Evidence suggests that kelts suffer substantial mortality during passage at mainstem hydropower facilities, in the estuary, and ocean (Evans 2002; Colotelo et al. 2012; Colotelo et al. 2013). Keefer et al. (2016) reported sea-to-sea survival of kelts from the Columbia and Snake rivers between 1% to 2%. They found kelt outmigration survival from the Snake River to below Bonneville Dam ranged from 13% to 20%. Kelt survival may also be limited by an increase in energy expenditure accompanying alteration of freshwater habitat (Love 1970; Penney and Moffitt 2014a). In the pre-dam era, repeat spawners were more prevalent, Long and Griffin (1937) reported 3.4% of scales from 1,128 steelhead collected in the lower Columbia River exhibited a spawn check. In contrast, iteroparity for upriver steelhead was estimated as 0.5% based on scales of 24,112 fish sampled at Bonneville Dam from 2004 to 2020 (Jeff Fryer personal communication). In less disturbed rivers such as the Nass and Skeena in British Columbia, contemporary repeat spawners make up an estimated 8% of the spawning run (Moore et al. 2014). In the Kalama River, an un-impounded tributary of the lower Columbia River below all mainstem hydropower facilities, it was documented that 17% of the run were repeat spawners in the 1990's (NMFS 1996).

In general, efforts to maintain or recover declining steelhead stocks within the Columbia River Basin have ranged from harvest reduction, mark-selective fisheries, habitat restoration, passage improvements (including transporting fish) at mainstem Columbia River hydro-power facilities, and hatchery propagation. These strategies have not recovered steelhead populations (Figure 1). Clearly, we need innovative approaches to obtain recovery of wild steelhead populations. Recently, the use of kelt reconditioning has been investigated as a novel method to increase total reproductive potential of steelhead populations (Hatch et al. 2013b). Kelt reconditioning was adopted as an offsite mitigation action in the 2008 Biological Opinion on the Federal Columbia River Power System as part of Reasonable and Prudent Alternative (RPA) 33 (NMFS 2008). The 2019 BiOp lists kelt reconditioning as an important strategy for wild steelhead recovery in the Columbia and Snake rivers (NMFS 2019).

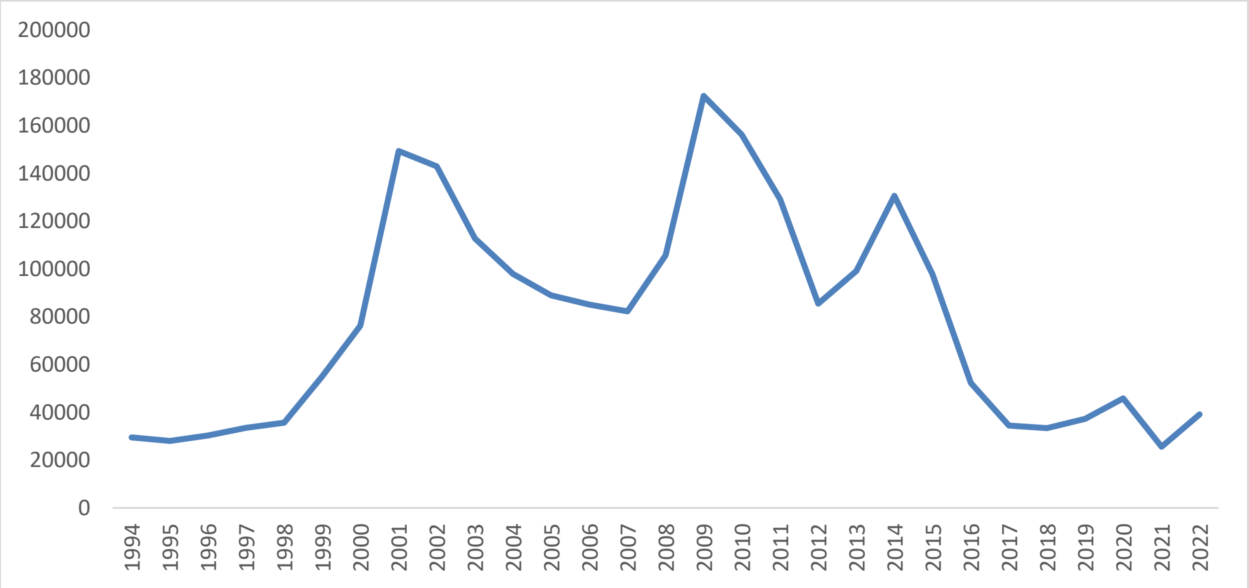


Figure I- 1. Annual counts of unclipped steelhead crossing Bonneville Dam.

The Kelt Reconditioning program is intended for application towards steelhead populations that have suffered declines in abundance and are targeted for restoration. In that context, an immediate boost in freshwater productivity is a means to offset long and short-term demographic perils and minimize loss of genetic and/or life history diversity. Preferably, kelt reconditioning programs should be accompanied by restoration or remediation targeting the root cause(s) of decline (Seamons and Quinn 2010). In evaluating restoration actions for steelhead, it should be noted, that the perils faced by iteroparous species may, or may not align perfectly with those faced by semelparous species, thus remediation targeting limiting factors for semelparous species may not be equally effective for iteroparous species. For example, habitat restoration actions have not explicitly focused on attributes that might increase kelt survival. Similarly, fish passage projects at mainstem hydropower facilities generally focus on upstream adult passage improvements, with far less emphasis on guidance and passage for emigrating adults, although this trend appears to be changing (Wertheimer et al. 2008). Thus, it is possible that existing restoration projects may be sufficient to secure demographic stability of a targeted population without simultaneously reestablishing historic rates of kelt contribution. Whether "asymmetric" recovery, as described above, is detrimental, has yet to be resolved, and is directly related to uncertainty about the genetic basis of iteroparity and its value as a life-history strategy.

It is unclear whether iteroparity is a genetic trait subject to loss within a population or Distinct Population Segment (DPS) versus a ubiquitous characteristic of the species whose expression is governed solely by environmental influence (i.e., a trait that cannot be "lost"; Seamons and Quinn 2010).

Several publications posit the theoretical benefits of iteroparity (Groot and Margolis 1991, Crespi and Teo 2002; Niemalä et al. 2006) and some document a realized increase in lifetime reproductive success accompanying iteroparity (Seamons and Quinn 2010); it is generally assumed that a loss or reduction in successful iteroparity simply results in a decrease in potential productivity. However, some recent evidence suggests that the loss of successful iteroparity can have profound impacts on not only productivity, but diversity, and population stability as well. In a recent study on British Columbia summer steelhead, Moore et al. (2014) found that increasing prevalence of repeat spawners reduced the magnitude of population fluctuations. Prevalence of repeat spawners had a larger effect on population stability than freshwater and ocean residence times, suggesting that there are substantial benefits of repeat spawners to population stability. Seamons and Quinn (2010) illustrate all these potential benefits. The authors studied a small population of coastal steelhead and found that on average, female repeat spawners had 1.9 times the reproductive success of females that spawned only once, while male repeat spawners produced nearly 2.7 times the number of adult offspring as males that spawned only once. Generally, these results support other empirical and theoretical observations regarding the potential benefits of iteroparity. Perhaps more importantly, the authors found that 71% of female repeat spawners typically produced the bulk of their surviving progeny in either the first or the second spawning event while only 29% of female repeat spawners successfully reproduced during two or more spawning events. Among repeat spawning males, none of the repeat spawners produced offspring during their first reproductive attempt; those that did successfully reproduce in their second attempt, produced nearly three times as many adult offspring as males that spawned on their first attempt. Although this is a single study of one population of steelhead, if generally applicable, the observations profoundly underscore the risks associated with a reduction in iteroparity; namely:

- Successful iteroparity can increase individual lifetime reproductive success by two to three times relative to semelparous individuals.
- A non-negligible fraction of male and female spawners fails to reproduce during their first spawning attempt, suggesting that genetic diversity could be directly reduced when conditions do not support successful iteroparity.

Aside from the documented benefits of iteroparity with regard to productivity, diversity, and demographic stability; reestablishment or enhancement of iteroparity has several potential management benefits:

1. Increased iteroparity improves fishery opportunities by increasing the total number and mean size of returning adults potentially vulnerable to fisheries.
2. Kelts can be reconditioned and held for use as broodstock, thus decreasing the demographic toll of broodstock collection on escapement for natural spawning.

3. Management actions to increase successful iteroparity may be implemented quickly and could achieve a demographic boost less controversially than artificial propagation and more quickly than habitat or passage improvements.
4. Reconditioning efforts can be focused on natural origin steelhead, thus substantially reducing chance for possible deleterious hatchery effects.

Significance to Fish and Wildlife Program and other regional plans

The principle of co-management is central to the Columbia Basin Fish and Wildlife Program, and the Northwest Power and Conservation Council is committed to working collaboratively with Tribes and in accordance with federal and state fish and wildlife laws and regulations (NPCC 2014). Sovereignty, and the legal rights of Tribes founded in treaties and tribes are understood to have governmental privileges over their lands, people, and natural resources. The United States has a trust obligation to protect the aforementioned, and the Northwest Power Act (NPCC 1980) aims to assist tribes in realizing their rights and responsibilities in regard to fish and wildlife.

Steelhead kelt reconditioning is part of the Technical Recommendation 4 Supplementation in Spirit of the Salmon: WY-KAN-USH-MI WA-KISH-WIT the tribes salmon recovery plan.

Steelhead kelt reconditioning programs in the Snake and Yakima rivers are included in NMFS recovery plans and in biological opinions:

- Page 192 and Appendix C Page 110 of the Snake River Steelhead Recovery Plan calls to implement the Kelt Management Plan and to continue to recondition Snake River steelhead kelts to benefit B-run populations. This project directly implements kelt reconditioning on B-run steelhead in the Snake River Basin.

- Page 2-1 and 2-6 in the Mid-Columbia steelhead recovery plan considers the Yakima River Kelt Reconditioning Program part of the DPS and MPG. Page 7-6 calls to develop and implement a kelt management plan. Page 7-9 recommends maintaining the existing kelt reconditioning program. Table 7-4 Recovery Strategies and Actions for the Yakima Basin MPG (page 7-35) lists the highest priority strategies that includes promoting repeat spawning of kelts, it affects all populations in the Yakima River, the key action would be using the kelt reconditioning program, it addresses all VSP parameters, and it addresses the reduced survival of kelts due to passage conditions in the Columbia and lower Yakima Rivers. Within the Yakima River specific recovery plan, Kelt Reconditioning is listed as a recovery strategy for all Yakima River populations (page 131) and continuing the program is listed as Basinwide Action #6 (page 148).

- The NMFS 2019 CRS Biological Opinion supports the Action Agencies to continue funding kelt reconditioning in the upper- and mid-Columbia as well as the Snake River on pages 43, 196 and 197.

- Snake River Kelt Management Plans and Progress Reports 2009-2018 (NOAA, BPA, COE documents 2009, 2011, 2013, 2015). Annual Management Plan and progress reports related to achieving the RPA 33 objective of increasing B-run steelhead in escapement in the Snake River. These include ongoing efforts, knowledge, management and research priorities within the framework of adaptive management. Page 24 of the 2015 report shows long term kelt reconditioning produced significantly higher rates of repeat spawners than other management strategies.

- The 2015 Snake River Basin Steelhead Kelt Reconditioning Facility Master Plan pages 16-24. Alterations to the Columbia River hydropower system have done little to improve the survival of emigrating steelhead kelts. As demonstrated, transport and short-term reconditioning appear to improve survival to the estuary but result in marginal increases in iteroparity rates. Therefore, we conclude that these strategies are unlikely to achieve

the survival benefits required by RPA 33. Available evidence suggests that long-term kelt reconditioning is the most promising kelt enhancement strategy for Snake River B-run steelhead to achieve RPA 33 which is a 6% increase in escapement in the Snake River.
- Yakima Basin Steelhead Reconditioning Project HGMP.

Steelhead kelt reconditioning guidance from the Council is found in many sources:

- Supporting the kelt steelhead reconditioning efforts in the Yakima and Snake river basins is stated as an accomplishment from implementation of the 2014 Fish and Wildlife Program in the 2020 Addendum, page 5.

The 2017 Research Plan and Critical Uncertainties document (NWPCC 2017) does not call out kelt reconditioning specifically but do provide guidance for assessing propagation programs. This guidance is useful and integrated into our program to assess kelt reconditioning in terms of population growth, abundance, spatial structure, and diversity as well as potential fitness impacts to the receiving population.

Goals and Objectives

The overall goal of the Kelt Reconditioning Project is to improve wild steelhead populations by increasing the abundance of natural repeat spawners. If this research points to a positive response, we plan to move to production level implementation of kelt reconditioning. To achieve this goal, we have identified the following goals, objectives, and milestones:

Goal 1. Develop techniques and strategies to collect, culture, care, and release wild kelt steelhead.

Objective 1.1 Evaluate fish collection locations and strategies to increase the abundance of kelt steelhead for reconditioning (designed capacity ~600 kelts) at the new Snake River Facility (once it is built).

Objective 1.2 Evaluate fish culture and care strategies to improve survival and rematuration above the long-term averages for each facility (~50%), continue until new facilities are built.

Objective 1.3 Continue to search for and evaluate fish characteristics at collection that correlate with survival and rematuration of artificially reconditioned kelts to achieve repeat spawners rates that are higher than the natural repeat rate in the receiving population (~3% in the Yakima and 0.5% in the Snake).

Objective 1.4 Evaluate strategies to manage skip spawners that optimize survival and spawning contribution, available space, and cost. This is planned to continue until new facilities are constructed.

Goal 2. Describe the benefits to the receiving population from releasing reconditioned kelt steelhead.

Objective 2.1 Evaluate receiving steelhead population abundance and growth and resulting contributions from released reconditioned kelts. This is planned to continue until new facilities are constructed.

Objective 2.2 Evaluate spatial structure and diversity of reconditioned kelts by tracking PIT detections throughout the basin. This is planned to continue until new facilities are constructed.

Goal 3. Develop and conduct evaluations of reproductive success and homing of reconditioned kelt steelhead.

Objective 3.1: Collect tissue samples from representative parent and offspring fish groups in the Yakima River through 2025.

Milestone 1. Completed annual juvenile collections through 2022.

Milestone 2. Completed annual adult collections through 2025.

Objective 3.2: Calculate relative reproductive success using parentage analysis from Yakima River collections from 2022-2025.

Milestone 1. Completed annual parentage analysis of juvenile samples.

Milestone 2. Submission of manuscript for reproductive success by juvenile assignments to a scientifically accredited journal by 2023.

Milestone 3. Submission of manuscript for adult-to-adult reproductive success to an appropriate journal by 2026.

Objective 3.3: Evaluate the homing and straying rates of reconditioned kelts in the Yakima River Basin. Monitor and analyze the detection history for verifiable first and second spawnings for all reconditioned kelts to estimate quantifiable homing and straying rates.

Milestone 1: Complete and submit a manuscript describing homing of reconditioned kelt steelhead to an appropriate journal by 2023.

Goal 4. Carry out studies to achieve a sufficiently detailed understanding of the physiology of reconditioning in kelt steelhead to provide a scientific basis for maximizing the success of reconditioning programs.

Objective 4.1: Use blood sampling and plasma reproductive hormone assays to evaluate reproductive status of kelts in reconditioning programs.

Milestone 1: Every year, collection of blood samples in September, assay of plasma estradiol or 11-ketotestosterone level, and assignment of each individual as a consecutive or skip spawner before fish are released in November.

Objective 4.2: Use physiological tools in studies to improve survival and rematuration rates at kelt reconditioning facilities (with Objective 1.2).

Objective 4.3: Use physiological tools to evaluate fish characteristics at collection that correlate with survival and rematuration at kelt reconditioning facilities (with Objective 1.3). If such predictors can be identified, they could be used to select fish for reconditioning.

Objective 4.4: Evaluate physiological indices at release as predictors of post-release migration and spawning success in kelts released from reconditioning programs. If such predictors can be identified, modifications to reconditioning practices can be tested to maximize post-release fish performance.

Goal 5. Project wrap up, delineation of necessary follow up monitoring and research, complete manuscripts, transition to other locations or uses of kelt reconditioning technology.

Objective 5.1: Develop plan for necessary monitoring and research to implement after kelt reconditioning production facilities at Prosser and Nez Perce Tribal hatcheries are completed and online.

Objective 5.2: Complete and submit manuscripts and technical reports related to kelt physiology, reproductive success, and homing from 2021-2023.

Milestone 1: Complete and submit manuscript describing kelt fasting experiment conducted using DNFH hatchery kelt model to a scientifically accredited journal by 2021-2022.

Milestone 2: Complete statistical analysis and writeup of study on post-spawning ration restriction using post-spawning rainbow trout model by 2021-2022, followed by submission to a scientifically accredited journal.

Milestone 3: Complete data processing, analysis and writeup of study on predictors of post-release migration and spawning success in female kelts release from Prosser Hatchery by 2022-2023. Submission to a scientific journal or publication as a technical report to depend on results.

Objective 5.3: Survey the region for potential to transfer kelt reconditioning technology to other locations and applications such as hatchery broodstock management or reintroductions.

An additional section with rationale for each goal, objective, and milestone is provided after the description of the study area.

Methods

A list of methods is provided in the **Appendix C**. This list provides direct hyperlinks to detailed project methods that are hosted on the **Monitoring Methods website**.

Study Area

Steelhead Kelt Collection, Reconditioning, and Release Sites

The study area is focused in the Yakima and Snake rivers. In the Yakima River, kelts are collected at the Chandler Juvenile Collection Facility (CJCF) at rkm 76 located downstream of all known steelhead spawning areas (Table 1 and Figure 2). The CJCF is situated on an irrigation canal where approximately 27% of the annual steelhead run are collected as kelts moving downstream. Kelts are transferred from the CJCF to the adjacent Prosser Hatchery for reconditioning. Juvenile collections for the relative reproductive success work are concentrated in Satus (rkm 27-62) and Toppenish (rkm 40-78) creeks.

In the Snake River, kelts are collected at the juvenile bypass screens at Lower Granite Dam (rkm 173), Little Goose Dam (rkm 113), Lower Monumental Dam (rkm 66) and experimented with collection in Asotin Creek using seine nets (rkm 233). These fish are trucked, then reconditioned at the Dworshak National Fish Hatchery, located at the confluence of the Clearwater and North Fork Clearwater rivers (rkm 65). Additional kelt rearing space is available at Nez Perce Tribal Hatchery (rkm 38), which is also the planned site of the future permanent Snake River kelt reconditioning facility (Hatch et al. 2021).

This project previously collected steelhead kelts at numerous sites around the Columbia River Basin. These sites are detailed in previous annual reports (Hatch et al. 2020, Hatch et al. 2015, Hatch et al. 2013a, Hatch et al. 2012, and Branstetter et al. 2008).

Table I- 1:Kelt steelhead collection, reconditioning, release, and juvenile collection sites used in this study. Site number corresponds with numbers in Figure 1.

Site Number	Site	Drainage	Location	Collection site	Reconditioning site	Release Site	Juvenile Sampling Location	Dates of use
1	Chandler Juvenile Collection Facility (CJCF)	Yakima River	Rkm 76	Yes	-	-	-	1999-2022
2	Yakama Nation Prosser Fish Hatchery	Yakima River	Rkm 76	-	Yes	Yes	-	1999-2022
3	Satus Creek	Yakima River	-	-	-	-	Yes	2008-2022
4	Toppenish Creek	Yakima River	-	-	-	-	Yes	2008-2022
5	Dworshak National Fish Hatchery (DNFH)	Clearwater River	Rkm 65	Yes, (hatchery fish for experimental purposes) 2009-2018	Yes	-	-	2009-2022
6	Nez Perce Tribal Fish Hatchery (NPTH)	Clearwater River	Rkm 38	No	Yes	-	-	2016-2019
7	Lower Granite Dam Juvenile Bypass (LGDJB)	Snake River	Rkm 173	Yes	-	Yes	-	2009-2022
8	Little Goose Dam Juvenile Bypass (LGSDJB)	Snake River	Rkm 113	Yes	-	-	-	2020-2022
9	Lower Monumental Dam (LMODJB)	Snake River	Rkm 46	Yes	-	-	-	2022
10	Asotin Creek.	Snake River	Rkm 233	Yes	-	-	-	2022

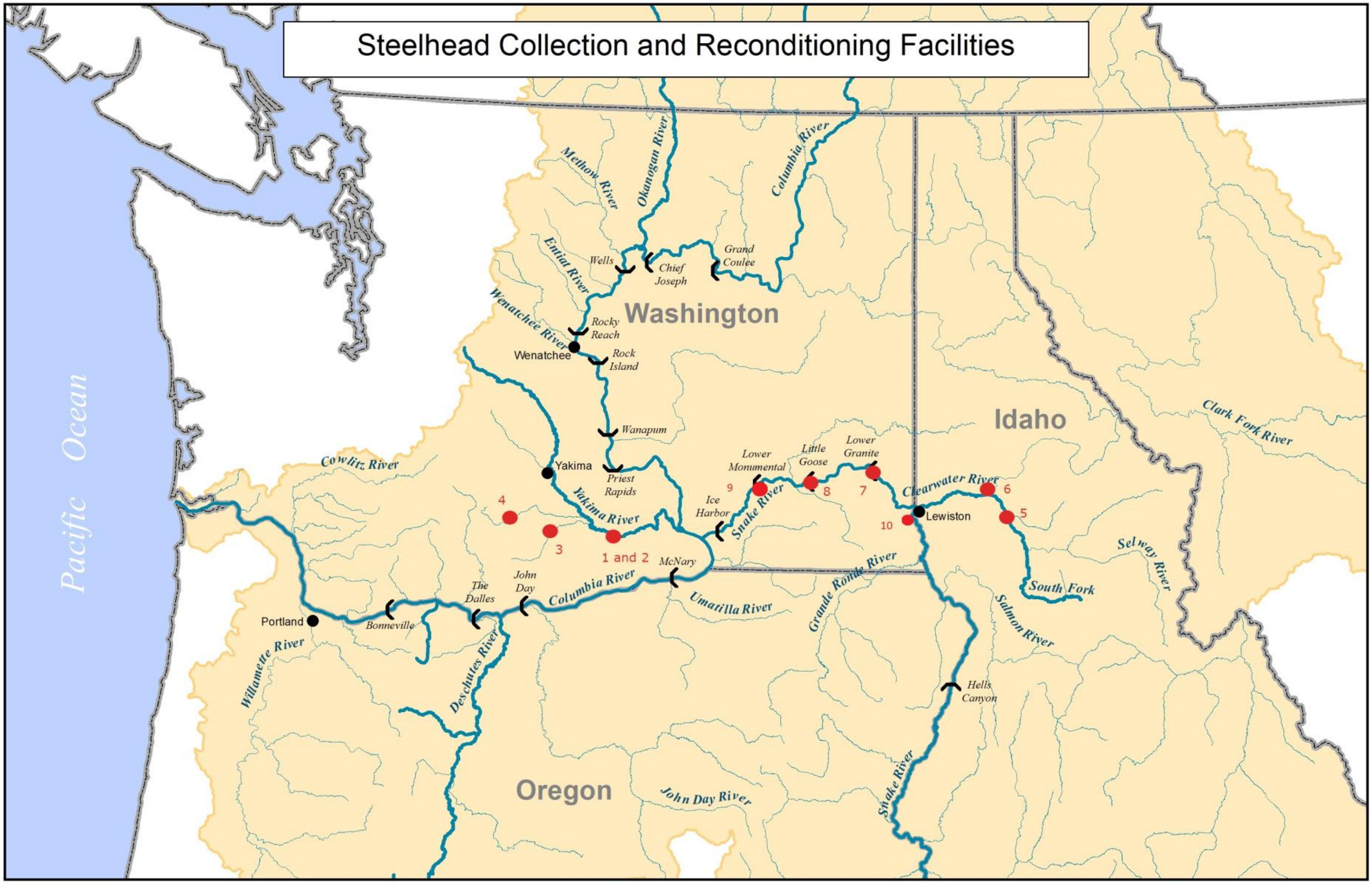


Figure I- 2: Map of Steelhead kelt Project area 2000-2022. Numbers correspond with site numbers in Table 1

Yakima River Basin

The Yakima River is approximately 344 km in length and enters the Columbia River at rkm 539. The basin is 15,928 km² and average discharge is 99 m^3/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995).

Chandler Juvenile Collection Facility (Yakima River)

Some post spawn steelhead (approximately 38%) migrating downriver are entrained in an irrigation canal and collected at the Chandler Juvenile Monitoring Facility (CJMF a.k.a. Chandler Juvenile Evaluation and Monitoring Facility CJEMF)) that screens migratory fishes away from the canal. The entire kelt collection for the Yakima River is conducted at the CJMF (Figure 2, Site1)

Yakama Nation Prosser Hatchery

Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam (rkm 75.6) and adjacent to the CJMF (Figure 2, Site 2). This facility is part of the The Yakima/Klickitat Fisheries Project, a supplementation project designated by the NPPC as the principle means of protecting, mitigating, and enhancing the anadromous fish populations in the Yakima and Klickitat Subbasins. Prosser Hatchery was constructed in 1994 with the primary function of rearing, acclimating, and releasing fall chinook salmon (*O. tshawytscha)*. It is also used for rearing coho salmon (*O. kisutch)* prior to acclimation and release in the upper Yakima River Basin as well as experimental rearing of white sturgeon *(Acipenser transmontanus)* and Pacific lamprey (*Entosphenus tridentate*).

Satus Creek.

Satus Creek Basin is located in south-central Washington (Figure 1, Site 3). Within the southeast portion of the Yakima Indian Reservation (Figure 1). Satus Creek enters the Yakima River at rkm 112. It is bounded on the north by Toppenish Ridge and by Horse Heaven Hills on the south. The western headwaters are contained by the Simcoe Range, while the eastern boundary is defined by the Yakima River (Hubble 1992). The basin has a drainage area of 612 square miles, which comprises 10% of the total Yakima Basin area. Many of the headwater streams in the Satus Creek watershed flow across plateaus bordered by wet meadows. As they flow generally eastward the headwater streams cascade through narrow canyons toward the relatively broad Satus Creek canyon. Satus Creek exits the canyon at RM 12.5 and flows eastward across the Yakima River floodplain to the confluence with the Yakima River. Most of the Satus Creek watershed is undeveloped and is not exposed to agricultural, industrial, or domestic effluents, but because of riparian conditions and low flow, maximum weekly average temperatures can exceed 26^o C in the reach of Satus Creek between Logy Creek and Wilson Charley Creek (rkm 63.2). Logy Creek cools Satus Creek for a few miles downstream from their confluence. Water quality suffers in this area, although water quantity increases as Satus Creek flows through the Wapato Irrigation Project (WIP) in its lower eight miles. The juvenile steelhead out-migration from Satus Creek consists of rapid and at relatively young age and appears to be a population response to the harsh summer conditions (YBFWRB 2009).

Toppenish Creek

The Toppenish Creek watershed, at 625 square miles, is similar in size to the Satus Creek watershed (Figure 1, Site 4) and is geographically the next watershed due north of Satus Cr. Toppenish Creek enters the Yakima River at rkm 120. The Upper Toppenish Creek consists of three forks along with several smaller tributaries draining the Cascade foothills between Toppenish and Ahtanum ridges. These are all high gradient streams in narrow basalt canyons. Anadromous access is limited by steadily increasing gradient and coarsening substrates, not the distinct waterfalls typical in the Satus Creek system. Agency and Wahtum creeks flow into Simcoe Creek, which joins Toppenish Creek at low elevation. A few miles downstream of the Simcoe Creek confluence, the Toppenish Creek channel historically assumed a branched appearance and flowed through an extensive network of wetlands for nearly 30 miles to the Yakima River. This network has a gradient of less than 0.1%. The complexity of this network has been significantly reduced from man-made interventions in stream flow (YBFWRB 2009). Most of the Yakama Nation's member population lives within this watershed (BoR 2018).

Snake River Basin

The Snake River watershed is the tenth largest among North American rivers and covers almost 280,000 km^2 in portions of six U.S. states: Wyoming, Idaho, Nevada, Utah, Oregon, and Washington, with the largest portion in Idaho. Most of the Snake River watershed lies between the Rocky Mountains on the east and the Columbia Plateau on the northwest. The largest tributary of the Columbia River, the Snake River watershed makes up about 41% of the entire Columbia River basin. The Snake River enters the Columbia at rkm 523. Its average discharge at the mouth constitutes 31% of the Columbia's flow at that point. The Snake River's average flow is 1,553 m^3/s. At Anatone, Washington, downstream of the confluences with the Salmon and Grand Ronde, but upstream of the Clearwater, the mean discharge is 979 m^3/s. Steelhead spawn naturally throughout the lower portion of the basin with the vast amount of "B-run" steelhead produced at the Dworshak National Fish Hatchery found on the Clearwater River.

The Lower Granite Dam Juvenile Bypass System

The fourth dam on the Snake River, the Lower Granite lock and dam, is a concrete gravity run-of-the-river dam on the Snake River, in the U.S. state of Washington. The dam is located 35 km (22 miles) south of the town of Colfax, and 35 miles (56 km) north of Pomeroy (Wikipedia). Steelhead kelts migrating from tributaries of the Snake River above Lower Granite Dam that do not emigrate via the Removable Spillway Weir (RSW) are directed by a large bypass system to the Juvenile Fish Facility (JFF) at Lower Granite Dam (LGR) (rkm 173) (Figure 2, Site 7).

The Little Goose Dam Juvenile Bypass System

The Third Dam on the Snake River, the Little Goose lock and dam, is a concrete gravity run-of-the-river dam on the Snake River, in the U.S. state of Washington. The dam is located 14km (9mi) northeast of the town of Starbuck, and 40km (25 mi) north of Dayton (Wikipedia). Steelhead kelts migrating from tributaries of the Snake River above Little Goose Dam that do not emigrate via the Removable Spillway Weir (RSW) are directed by a large bypass system to the Juvenile Fish Facility (JFF) at Little Goose Dam (LGS) (rkm 113).

The Lower Monumental Dam Juvenile Bypass System

The second Dam on the Snake River, the Lower Monumental lock and dam (LMO), is a concrete gravity run-of-the-river dam on the Snake River, in the U.S. state of Washington. The dam is located it is six miles (10 km) south of Kahlotus and 43 miles (70 km) north of Walla Walla (Wikipedia). Steelhead kelts migrating from tributaries of the Snake River above Little Goose Dam that do not emigrate via the Removable Spillway Weir (RSW) are directed by a large bypass system to the Juvenile Fish Facility (JFF) at Lower Monumental Dam (LMO) (rkm 46).

Asotin Creek

The Asotin Creek Subbasin is located in the southeast corner of Washington and drains about 84,000 hectares of the northeast corner of the Blue Mountains. Asotin Creek is a third order tributary of the Snake River, joining it at the town of Asotin (Figure 2, Site 10). Asotin Creek has two major watersheds: The mainstem and George Creek. The mainstem (above George Creek) drains about 48,000 hectares (118,000 acres) and is the area of focus for this project. Major tributaries of the mainstem include Charley Creek, North Fork, South Fork, and Lick Creek. George Creek drains about 36,000 hectares (89,000 acres) (Mayer et al 2008).

Dworshak National Fish Hatchery

Kelt reconditioning facilities are located at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho. DNFH is located at the confluence of the North Fork of the Clearwater River (rkm 65). The Dworshak National Fish Hatchery is a "mitigation" hatchery constructed in 1969 by the Army Corps of Engineers, which presently is co-managed by the U.S. Fish and Wildlife Service and the Nez Perce Tribe (USFWS 2009). Kelts from Lower Granite and hatchery origin fish have been reconditioned at this facility since 2009. Beginning in 2016 most of the kelts reconditioned at this location were hatchery fish that returned to the hatchery. They are then air spawned and reconditioned to learn more about kelt rematuration and how we can improve maturation and survival. Through 2019 besides the experimental groups surplus Lower Granite

Dam captured “wild” kelts were held on site and trucked to NPTH in the fall. In both 2020 and 2021 all the Lower Granite Dam and Little Goose kelts collected and selected for reconditioning were trucked and reconditioned at this location due to technical issues (water sourcing) at NPTH.

Nez Perce Tribal Hatchery

Starting in mid-2016 kelt reconditioning tanks were established at the Nez Perce Tribal Fish Hatchery site situated at Nez Perce Tribal allotment site 1705, located 38 km above the mouth of the Clearwater River. This Nez Perce Tribe managed facility was constructed in 2002 and was primarily used to supplement spring and fall chinook (*O. tshawytscha*) in the Clearwater River. Most steelhead kelts captured at Lower Granite Dam are trucked here to be reconditioned. No fish were reconditioned here in 2020 through 2022 due to technical issues that need to be resolved before reconditioning can resume (Hatch et al 2020). This is the future site of a permanent steelhead kelt reconditioning facility which should resolve the water sourcing issues.

Goal 1. Develop techniques and strategies to collect, culture, care, and release wild kelt steelhead.

Goal 1, Objective 1.1: Evaluate fish collection locations and strategies to increase the abundance of kelt steelhead for reconditioning (designed capacity ~600 kelts) at the new Snake River Facility (once it is built and operational; scheduled 2023) and in the Yakima River.

We have operated kelt reconditioning at a research scale by collecting fish at the Chandler Juvenile Collection Facility (CJCF) and Lower Granite Dam. Production scale facilities with larger capacities may require collections at additional locations to obtain enough fish to fill the new facilities. To locate suitable collections sites, we plan to:

- Develop collection site criteria: access to kelts, abundance of kelts, ability to hold kelts prior to transport to reconditioning facility, potential conflicts with other programs, and other considerations.
- Consult co-managers and maps for potential collection sites and conduct site visits.
- Develop implementation plans for additional collection sites.

Goal 1, Objective 1.2: Evaluate fish culture and care strategies to improve survival and rematuration above the long-term averages for each facility (~50%), continue until new facilities are built.

Kelt steelhead are collected from the separator at juvenile bypass screens by dipnetting and transfer to a temporary holding tank containing oxygenated well water (13.8◦C). Each fish is anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 600 µL/L, weighed, measured for FL, and judged by experienced fish culture staff as to maturation status, sex, condition (good = 1; fair = 2; poor = 3) and coloration (bright = 1; intermediate = 2; dark = 3). Condition is based on the degree of visible external damage (e.g., abrasions, lesions, fungal infections; see Evans 2003), and coloration (an indicator of physiological state) was based on the degree of the fish's silvery, ocean-like external appearance. A uniquely coded PIT tag is injected into the pelvic girdle of each kelt (Prentice et al. 1990) for individual fish identification during reconditioning and post release tracking.

Reconditioned kelts are fed a combination of frozen krill and 6.0-mm pellet feed manufactured by Bio-Oregon. Krill is used initially to enhance the feeding response, and after 4–6 weeks the pellets are introduced. Feed is administered 3–5 times daily at a rate of 1–2% body weight or until fish seemed satiated. The tanks were covered to provide shelter from sun and relieve stress from outside movement. Any kelt mortalities were removed daily and the tanks were swept and flushed every 10–14 d as needed. The tank walls were painted white and the centers dark to discourage the kelts from rubbing the walls. We found eye damage became prevalent when fish excessively rubbed the walls (Hatch et al. 2013a). Aerators were placed in the tanks to break up the surface and introduce oxygen. The aerators appear to reduce stress by providing added security for the fish.

Before their release from the reconditioning facility, surviving steelhead are again weighed, measured (FL), sexed, and scanned for PIT tags. Fish are released from mid- October to early December, concurrent with the peak return of the natural spawning run. Releases are made downstream of both Prosser and Lower Granite dams for Yakima and Snake rivers origin fish, respectively. The release location and timing allow reconditioned kelts to naturally select their migration timing, spawning location, spawn timing, and mates.

Our existing fish care protocols (Hatch et al. 2013a) are achieving survival rates to the first fall around 50%. We plan to continue refining these protocols calculating and tracking survival, rematuration and making comparisons among locations described in Hatch et al. (2021).

Starting in 2022, we switched from Parasite-S to a 35% Perox-AID (hydrogen peroxide) product from Syndel to control fungus at Prosser. Treatments will be administered at the same rate, duration, and utilize the same delivery method as the Parasite-S treatments. All fish in this treatment will receive every-other day treatments from May through release in October. We hope to have all fish on this treatement but some permitting issues will need to be done at Dworshak before we can proceed.

At Dworshak National Fish Hatchery (DNFH) we are still using Parasite-S (Formalin) from Syndel to control fungus while reconditioning steelhead kelts. The fungicide is delivered to the individual tanks with a MasterFlex L/S peristaltic pump and C-Flex Tubing LS/25 from Cole-Parmer. The tubing is housed in 3/4" PVC from the chemical storage room to the point of delivery in the tanks. The tanks are 15' round, water depth of 4', and an inflow of 60-65gal/min. The base treatment used is a flow-through treatment of 23.5ml/min of Parasite-S for 2hrs 46min. All incoming kelts receive daily treatments for approximately 6 months. At this point, the kelts are either released or held as skip spawners. All skip spawning kelts receive a treatment every-other day until they are released the following year.

Goal 1, Objective 1.3: Continue to search for and evaluate fish characteristics at collection that correlate with survival and rematuration of artificially reconditioned kelts to achieve repeat spawner rates that are higher than the natural repeat rate in the receiving population (~3% in the Yakima and 0.5% in the Snake).

All captured steelhead are scanned for existing PIT-tags, and biological data is collected as described above. To evaluate the change in fish form as a result of reconditioning, we calculate Fulton's condition factor (K) at collection and at release (Ricker 1975). We use the calculation K = w/l, where w = fish weight (kg) and l = fish length (cm). One-way analysis of variance (ANOVA) is used to determine differences, at collection, in kelt length, weight, condition, and color across years (Sokal and Rohlf 2000). We use ANOVA tests to evaluate the association of survival (0, 1) with several fish trait variables, including FL, weight, K, condition, and color. Additionally, we calculate correlation coefficients among fish variables along with annual abundance of kelt and prespawn steelhead, the proportion that kelt steelhead made up of the previous run, length and weight change, and standardized flow periods (Sokal and Rohlf 2000).

Goal 1 Objective 1.4: Evaluate strategies to manage skip spawners that optimize survival and spawning contribution, available space, and cost. This is planned to continue until new facilities are constructed.

Long-term reconditioning is a management strategy where emigrating kelt steelhead are collected and held in large tanks, given prophylactic treatments and fed a specially formulated diet for approximately 6 months (Hatch et al. 2013a). After 6 months, the "reconditioned" kelts are released back into the collection river as the run at large is returning from the ocean. These reconditioned fish generally mingle with the run at large and proceed to in-river, over-winter locations and spawning grounds in the spring. We have determined that both natural and artificially reconditioned kelts can pursue two alternative pathways toward rematuration and repeat spawning. One pathway is termed consecutive spawning where individuals remature and proceed to spawn in the next spawn cycle. The other pathway is termed skip spawning where individuals remature and proceed to spawn two years after their previous spawning. To illustrate, kelts collected in the spring of 2020 could spawn again in the winter/spring 2021 as consecutive spawners or wait until spring of 2022 and spawn as skip spawners. The proportion of consecutive and skip spawners in a cohort varies annually, but in general, Yakima River fish predominately follow the consecutive spawner pathway (60-70%) and will spawn the following winter/spring, while the majority of Snake River kelts follow the skip spawner life history (60-70%). Strategy choice may be controlled by a combination of genetics and environment, while also varying from year to year. We plan to develop and evaluate alternative management strategies for skip spawners.

Our skip spawner management approach in the Snake River has been to hold the fish in the hatchery for an additional 12 months. This has been very successful in terms of survival, >85%

for the second year. In the Yakima River, the Prosser Hatchery kelt tanks operate on well water that in the winter is much warmer than the river. This relatively warm water disrupts the spawning schedule of skip spawners held for the additional 12 months. The Prosser Hatchery Master Plan calls for a recirculating / chilled water system to allow us to successfully hold skip spawners over the winter, but in the meantime, we are searching for an alternative skip spawner strategy for Yakima River kelts until the new facility is constructed.

Skip spawner management strategies to evaluate include:

1. Rear for an additional 12 months in the hatchery. This is the current Snake River approach and the survival and rematuration rates will be used as the baseline values to evaluate other alternatives.

2. In the fall at the time when consecutive spawners are released, transport Yakima River fish that are on skip spawner trajectory to the lower Columbia River and release. Return rates, based on PIT detections through the hydrosystem will used as an index of repeat spawner rates and compared against strategy 1. Based on recent steelhead returns to the Yakima River, we anticipate a sample size of 75 to 100 fish in the transport group. We have discontinued attempting to use this approach as we did not see any fish return from the ocean.

3. To increase the sample size of the transport group, we plan to explore other sources of reconditioned kelt steelhead for the transport test. A possible, yet currently unexplored, source of kelts would be Dworshak Hatchery broodstock (or some other steelhead hatchery). If these fish were available, they could be air spawned in the spring, held, and reconditioned until fall, transported (for skip spawners), and evaluated by PIT detection on their return. The sample size for this group could be approximately 200 fish. This experiment is merely a possibility and will require extensive coordination with co-managers including the Fish and Wildlife Service.

Goal 2. Describe the benefits to the receiving population from releasing reconditioned kelt steelhead.

Goal 2. Objective 2.1: Evaluate receiving steelhead population abundance, growth, and resulting contributions from released reconditioned kelts. This is planned to continue until new facilities are constructed.

We plan to continue to evaluate the contribution of reconditioned kelts to receiving populations. Evaluations in the Yakima River will include ocean return steelhead counts at Prosser Dam, the number of mature reconditioned kelts released in October, and tracking the proportion of the run that is comprised of reconditioned kelts. We plan to continue tracking the proportion of the wild group A steelhead run at Bonneville Dam that is made up of Yakima River fish. The proportion will be calculated from counts of wild group A steelhead at Bonneville Dam and steelhead counts at Prosser Dam.

We will use a life cycle model to predict the effect of reconditioning and releasing a range of proportions of kelts. The predictions will account for the condition of released kelts, and the resulting probability of successfully spawning. Progeny will be assumed to contribute to the naturally spawning population. A sensitivity analysis of the rate of contribution of kelts to naturally spawning steelhead will be limited to the capacity of the reconditioning facility such that the analysis can account for a variable facility capacity.

Goal 2, Objective 2.2: Evaluate spatial structure and diversity of reconditioned kelts by tracking PIT detections throughout the basin. This is planned to continue until new facilities are constructed.

The growing network of in-stream PIT tag arrays provide an opportunity to track reconditioned kelts to many spawning locations in the Snake and Yakima rivers. We plan to use this network to characterize the spatial distribution of kelts, and to map the contribution of reconditioned kelts to abundances in streams all over the basin.

An annual record of kelt detections at each PIT tagged stream will be maintained, providing an annual record of the proportion of reconditioned kelt releases arriving at each receiving population stream. The record will also provide an indication of the proportional contribution of reconditioned kelts to each stream's spawning relative to naturally spawning maidens. The proportional distribution of kelts (spatial variability) across all receiving populations will provide an indication of the spatial diversification effect of kelt releases.

Goal 3. Develop and conduct evaluations of reproductive success and homing of reconditioned kelt steelhead.

Goal 3, Objective 3.1: Collect tissue samples from representative parent and offspring fish groups in the Yakima River through 2025.

Anadromous adult steelhead will be collected as either upstream migrants at Prosser Dam or downstream migrants at the Chandler Juvenile Collection Facility. Samples collected as upstream migrants at Prosser Dam will be treated as maidens and referred to as pre-spawn maiden collections. Post-spawn adults sampled at the Chandler facility that did not go into the reconditioning program or that did not survive the reconditioning program are referred to as post-spawn maidens. Adults collected at the Chandler facility and reconditioned prior to release in the fall are referred to as kelts.

Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) will be targeted using electrofishing techniques (NMFS 2000 Electrofishing Guidelines) during the late summer and fall in the Satus and Toppenish tributaries. Additional samples from rotary screw traps may be included. Sampling will be targeted near areas where steelhead spawning has been observed or previous sampling efforts have provided progeny with anadromous parents. Technicians in the field will target age-0 juveniles with a general maximum length of 100mm in addition to the judgment of those collecting the samples based on the time of collection. Individual Fork length will be recorded.

Milestone 1. Completed annual juvenile collections through 2022.

Milestone 2. Completed annual adult collections through 2025.

Goal 3, Objective 3.2: Calculate relative reproductive success using parentage analysis from Yakima River collections from 2022-2025.

Genetic analysis will be conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA will be extracted from tissue samples using chelex beads and genotype data generated with a lab standardized panel of SNP markers using a targeted amplicon GT-seq method on a next generation sequencer. Prior to parentage analysis, a sex-determining marker (OmyY1_2SEX), three loci diagnostic for cutthroat, poor performing loci and loci with low minor allele frequency will be dropped. Unresolved duplicate samples, samples with incomplete genotypes, and non-target species samples will be omitted.

Parentage analysis will be performed using CERVUS v 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). Information on fish gender is not included in the analysis. To minimize incorrect assignments, simulations will be performed annually to estimate a 99.0% confidence LOD value. Individual parentage assignments will be included if they have a minimum of 90% loci comparisons, meet the critical LOD value, and have minimal single locus mismatches. This should account for the presence of minor genotyping errors while minimizing the loss of parental assignment matches.

Relative reproductive success (RRS) will be calculated annually between classes of fish by standardizing to the pre-spawn maiden class of adults. Lifetime reproductive success (LRS) estimates for kelts will be calculated by adding the RRS of Event-1 and Event-2 estimates. This estimate of LRS does not look at individual fish that spawned across multiple years, nor does it look at the same group of fish across 2 consecutive years (e.g., Maiden in 2013, reconditioned kelts in 2014). Rather, it adds the RRS estimates of fish spawning in the same calendar year.

Milestone 1. Completed annual parentage analysis of juvenile samples.

Milestone 2. Submission of manuscript for reproductive success by juvenile assignments to a scientifically accredited journal by 2023.

Milestone 3. Submission of manuscript for adult-to-adult reproductive success to an appropriate journal by 2026.

Goal 3, Objective 3.3: Evaluate the homing and straying rates of reconditioned kelts in the Yakima River Basin. Monitor and analyze the detection history for verifiable first and second spawnings for all reconditioned kelts to estimate quantifiable homing and straying rates.

Sub-basin level homing of reconditioned kelt steelhead

In the Yakima River, kelt steelhead are reconditioned and released in the fall approximately four months prior to the next spawning season, but not all fish are appropriate for a repeat homing fidelity investigation since the released population in the past contained both mature (consecutive) and immature (skip) spawners. All long-term reconditioned kelts for this study will be released below Prosser Dam in the lower Yakima River with a PIT tag for individual identification. Final spawning destination of these individuals will be assigned through tributary, in-river, or dam PIT tag detection, recapture prior to spawning, or recollection as post spawn kelts the following emigration season. If the reconditioned kelts return to the same river system they were collected prior to or during the subsequent spawning season, then we will presume that they have rehomed to the same river basin they spawned as maidens. If they are detected in a sub-basin other than the one they were collected in, prior to, or during the subsequent spawning season, then we will presume that they strayed from the location they spawned in as maidens.

It will not be possible to determine the final destination for all fish released below Prosser Dam, therefore, we will exclude all fish that are not detected post release (likely lost tag or immediate mortalities), fish that are likely immature or skip spawners that are either lost to the Mainstem Columbia River hydrosystem during their emigration, or fish that emigrate successfully but do not successfully reascend to a sub-basin river, and have a subsequent spawning season (likely harvest or hydrosystem mortalities). Final PIT tag detections will be determined using the PTAGIS database. Once the steelhead spawning season concludes each year, the PTAGIS database will be queried to obtain the complete tag history for all PIT tag codes released from the kelt reconditioning program the previous fall (MethodID: 4095).Final detections will be assigned using the last uppermost detection location which includes in-river or dam PIT arrays, and/or recapture from sampling programs throughout the basin.

Sub-population level homing of reconditioned kelt steelhead

To investigate smaller scale sub-population level homing fidelity, we will limit our investigation to specific known sub-population maiden spawners and subsequent reconditioned kelt spawner homing events. Maiden spawner origin will be determined through detections of first-time spawners outfitted with PIT tags at tributary level or sub-population detection arrays, through parentage analysis of progeny collected in tributaries that connect confirmed maiden spawning locations to kelt steelhead parents in the reconditioning program, or kelt steelhead collected at tributary weirs.

Installation of in-stream PIT arrays provide data at both the individual and stock level. PIT arrays are installed in Satus Creek, Toppenish Creek, Ahtanum Creek, Taneum Creek, and Roza Dam in the Yakima River Basin, and in Omak Creek in the Okanogan River Basin. Wild origin steelhead are rarely PIT tagged, therefore the number of maiden spawners that will be previously PIT tagged and detected at the sub-population level during maiden spawning events is relatively low. To increase sample size at the maiden stage, we will use parentage results as a proxy for PIT tag detections of the maiden spawning event. Satus and Toppenish creeks have an ongoing reproductive success study which permits the assignment of Age-0 juveniles to kelt parents in the reconditioning program (see ***Goal 3, Objective 3.1*** for methods), thereby identifying the parents of these juveniles from the maiden spawning event. Likewise, previously PIT tagged wild origin steelhead in Omak Creek are rare, but all the wild steelhead kelts collected for the reconditioning program originated from the Omak Creek weir, therefore, confirming the origin of their first spawning.

All reconditioned kelts used in this portion of this study will be PIT tagged and released either below Prosser Dam in the Yakima River or in the mainstem Okanogan River approximately four months prior to the subsequent spawning season. Like the Sub-Basin level study, PIT tagged reconditioned kelts that could not be tracked for the reasons outlined above will be excluded from the analysis. If reconditioned kelt steelhead are detected at PIT tag arrays of their maiden tributary a second time in a subsequent spawning season, we will consider that successful homing. If reconditioned kelts return to a different tributary, then we will consider those fish to have strayed. Fish included in this study will not be included in the sub-basin level study.

Milestone 1: Complete and submit a manuscript describing homing of reconditioned kelt steelhead to an appropriate journal by 2023.

Goal 4. Carry out studies to achieve a sufficiently detailed understanding of the physiology of reconditioning in kelt steelhead to provide a scientific basis for maximizing the success of reconditioning programs.

Goal 4, Objective 4.1: Use blood sampling and plasma reproductive hormone assays to evaluate reproductive status of kelts in reconditioning programs.

Kelts will be sampled by either project leaders or the physiology team in September of each year. Non-lethal blood sampling of kelts will be conducted by caudal venipuncture according to published methods (Caldwell, et al. 2013; Houston 1990; Jenkins, et al. 2019; Pierce, et al. 2017). The volume of blood taken is a small fraction of the total, and the sampling procedure is not particularly hard on the fish. Approximately 2-mL of blood will be drawn from anesthetized fish using heparinized syringes, centrifuged, and plasma stored at -80 ^{O}C prior to assay for estradiol (E2) in females or 11-ketotestosterone (11-KT) in males. E2 is the principal estrogen and 11-KT the principal androgen in fishes. An extraction procedure to remove interfering substances is required before steroid assays (MethodID: 5319). Standard clinical assays designed for human or mammalian plasma will not give accurate results without extraction. Extraction and assay procedures will be conducted at the CRITFC/University of Idaho physiology laboratory according to published methods (Caldwell, et al. 2014; Jenkins et al. 2019; Medeiros, et al. 2018)(MethodID: 371, MethodID: 5320). A histogram of log plasma E2 or 11-KT levels will be constructed and the cutoff between maturing and non-maturing individuals identified. This is typically about 1000 pg/ml E2, although it may vary depending on sampling time and the kelt population. Less information is available on males, but a cutoff of 2000-4000 pg/ml 11-KT is typical.

Goal 4, Objective 4.2: Use physiological tools in studies to improve survival and rematuration rates at kelt reconditioning facilities (with Objective 1.2).

Blood sampling, plasma hormone assays, and other plasma factor assays will be used to assess recovery from spawning, growth and metabolic status, and reproductive development in kelts during reconditioning. Blood sampling and reproductive steroid assays will be conducted as described above (Goal 4, Objective 4.1). Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) assays will be conducted as described in a recent publication from our laboratory

(Medeiros, et al. 2020). Plasma metabolite and osmolality assays will be conducted as previously described (Jenkins, et al. 2020; Jenkins et al. 2019).

Goal 4, Objective 4.3: Use physiological tools to evaluate fish characteristics at collection that correlate with survival and rematuration at kelt reconditioning facilities (with Objective 1.3).

Blood sampling of fish at collection for reconditioning and plasma assays will be conducted as described above (Goal 4, Objective 4.2). The survival and maturation status for each fish are tracked as part of the standard operation of reconditioning facilities. Multiple logistic regression analysis will be employed to determine predictors of survival and rematuration (e.g., Jenkins et al. 2020).

Goal 4, Objective 4: Evaluate physiological indices at release as predictors of post-release migration and spawning success in kelts released from reconditioning programs.

Blood sampling of fish during the fall survey for maturation status or at release and plasma assays will be conducted as described above (Goal 4, Objective 4.2). Migration and spawning success will be assessed by post-release PIT tag detection histories as described above (Goal 3, Objective 3.2). Multiple logistic regression analysis will be employed to evaluate predictors of migration and spawning success.

Goal 5. Project wrap up, delineation of necessary follow up monitoring and research, complete manuscripts, transition to other locations or uses of kelt reconditioning technology.

Goal 5, Objective 5.1: Develop plan for necessary monitoring and research to implement after kelt reconditioning production facilities at Prosser and Nez Perce Tribal hatcheries are completed and online.

This project has operated at a research-scale to prove the concept of kelt reconditioning. That phase will end once dedicated kelt reconditioning facilities are constructed and online. We will develop a plan for monitoring and evaluating the production-scale facilities. In addition, we will identify any research topics that require further study (I.e., skip spawner management strategies).

Goal 5, Objective 5.2: Complete and submit manuscripts and technical reports related to kelt physiology.

We have several studies and experiments that need to be fully analyzed, written up, and published. This work is ongoing and will extend into 2023.

Milestone 1: Complete and submit manuscript describing kelt fasting experiment conducted using DNFH hatchery kelt model to a scientifically accredited journal in 2022 (Please see Jenkins et al. 2022 in Appendix B).

Milestone 2: Complete statistical analysis and writeup of study on post-spawning ration restriction using post-spawning rainbow trout model by 2023, followed by submission to a scientifically accredited journal.

***Milestone 3*:** Complete data processing, analysis and writeup of study on predictors of post-release migration and spawning success in female kelts release from Prosser Hatchery by 2022-2023. Submission to a scientific journal or publication as a technical report to depend on results.

Goal 5, Objective 5.3: Survey the region for potential to transfer kelt reconditioning technology to other locations and applications such as hatchery broodstock management or reintroductions.

We plan to survey the region for potential applications of kelt reconditioning in other sub basins and applications of kelt reconditioning. Potential applications could be for hatchery broodstock management using air spawning technics and then reconditioning those fish for use in future spawning years as a hedge for years with inadequate broodstock. Wild reconditioned kelts could also be used as stock for reintroductions without impacting other fish sources. This work is planned to occur from 2022-2026.

Chapter 1: Kelt Capture, enumeration, and reconditioning in the Yakima and Snake basins.

Introduction

Kelt steelhead reconditioning process evaluations involve fish culturing practices, studying alternative management strategies, and implementing research scale reconditioning programs. Adding repeat spawner steelhead to the population through reconditioning can add stability through the portfolio effect (Moore et al. 2014) and increase population abundance by increasing lifetime reproductive success (Seamons and Quinn 2010). We established "control" groups in both the Snake and Yakima rivers. These control groups were downstream migrating kelts, systematically collected, PIT tagged and released back into the river each year. These fish are monitored through PTAGIS to determine how successfully they naturally recondition in the ocean.

We define long-term reconditioning as holding and feeding post-spawn steelhead in a captive environment to increase kelt survival and create additional spawning opportunities. The long-term steelhead reconditioning diet and care treatments were established from the studies conducted in 2001 and 2002 (Hatch et al. 2002 and Hatch et al. 2003b) and summarized in Hatch et al. 2013b. These fish are typically released in the fall to over-winter and return to the spawning sites volitionally. This chapter recaps our 2022 kelt collection efforts; for a broader review of specific fish culturing practices please see (Hatch et al. 2015).

Methods

Standard Data Collection

All captured steelhead are scanned for existing PIT-tags, and biological data is collected which includes determination of kelt/maiden status, fork length, weight, condition factor (color and presence/absence of wounds/skin-body condition), coloration rating (bright, medium, dark), notation of clipped or non-clipped fins (typically adipose), and small (typically a 1 x 1 mm) tissue sample (caudal fin clip) is collected for genetic analysis. Steelhead without a PIT-tag receive a 12.5 mm PIT-tag injected into the pelvic girdle to track migration history and to determine reconditioning efficacy. All releases or mortalities are recorded including date of event, condition factor, and PIT-tag identifier. In the case of a lost PIT-tag, typically at time of release, fish are retagged, and an additional genetic sample is collected. The data is then uploaded to a central kelt database at the CRITFC and any fish that are released back to the wild are entered into the PTAGIS database. Kelt detection movements are monitored by querying PTAGIS for both the Yakima and Snake rivers releases. These detection histories provide us evidence of kelt homing to natal stream. They sometimes reveal kelts that have managed to spawn on occasion a 3rd time.

Steelhead Kelt Collection

Chandler Juvenile Monitoring Facility

Once diverted into the CJMF (Table 1, site 1), emigrating kelts are manually collected from a fish separation device (a device that allows smaller juvenile salmonids to “fall through” for processing in the juvenile facility while larger fish can be dip netted for processing and transferring to the reconditioning tanks at Prosser Hatchery (Table 1, site 2). Yakama Nation staff monitored the Chandler bypass separator during the kelt migration.

Lower Granite Dam

Steelhead kelts entering the juvenile bypass separator (Table 1, site 7) are collected by Army Corps of Engineer (COE) staff. Kelts are netted off the adult fish separator bars and moved to a fish hopper that leads into the kelt receiving tank. Staff from the Nez Perce Tribe (NPT) processed fish that were diverted into the receiving tank. Kelt steelhead, judged to be in good or better condition, with intact adipose fins, and >63cm are then trucked to DNFH for reconditioning. The transport truck had a 1.5-kiloliter tank fitted with supplemental, regulated, and compressed oxygen that was fed via air stones; also, a 12-volt powered tank aeration pump was used to circulate oxygenated water. Stress Coat® or PolyAqua® was used to replace the natural protective slime coating that may have been compromised by handling. In addition, salt was added to reduce osmo-regulatory stress. Temperature and dissolved oxygen levels were also monitored during transport. Loading densities were kept to a minimum so that no more than 20 kelts were transported at one time.

Little Goose Dam

Collection and protocols followed are the same as Lower Granite Dam (Table 1, Site 8).

Lower Monumental Dam

Collection and protocols followed are the same as Lower Granite Dam (Table 1, Site 9)

Asotin Creek

Collection was conducted at a two way weir, operated by Washington Department of Fish and Wildlife (WDFW) and were placed in travel tank for transport to the Clarkston office. Collections were then transferred to Nez Perce Tribal staff and transported to DNFH (Table 1, Site 10).

Long-term Reconditioning

Long-term reconditioning is a management strategy where emigrating kelt steelhead are collected and held in large tanks, given prophylactic treatments and fed a specially formulated diet for approximately 6 months (Hatch et al. 2013b). After 6 months, the “reconditioned” kelts are released back into the collection river as the run at large is returning from the ocean. These reconditioned fish generally mingle with the run at large and proceed to in-river, over-winter locations and spawning grounds in the spring. This strategy seeks to reduce mortality in the hydro system and ocean, providing another opportunity for fish to reproduce in the wild. Techniques used in kelt reconditioning were initially developed for Atlantic salmon *Salmo salar* and Brown or Sea-trout *S. trutta*, and a review of these studies and others applicable to steelhead kelts are summarized in Evans et al. (2001). All kelt reconditioning conducted at Prosser Hatchery is primarily done in 4, 6.1m x 1.5m (20' x 5') circular tanks, 4 -smaller, 3m x 1.2m (10' x 4'), and one, 5.2m x 1.5m (17' x 5') circular tank also available. The water is spring fed, at 10-11^{0}C. Fish are held at DNFH in one of 4, 4.6m diameter outdoor tanks, supplied with North Fork Clearwater River water at 200 liter/minute, maintained at a water height of 1.5m, with a seasonally varying temperature profile of 4.9 – 11.0^{o}C.

Life History Strategies: Consecutive vs. Skip Spawning

The steelhead reproductive physiology research conducted by the CRITFC has determined that both natural and artificially reconditioned kelts can pursue two alternative pathways toward rematuration and repeat spawning. One pathway is termed consecutive spawning, where individuals remature and proceed to spawn in the next spawn cycle. The other pathway is termed skip spawning, where individuals remature and proceed to spawn two years after their previous spawning. To illustrate, kelts collected in the spring of 2022 could spawn again in the winter/spring of 2023 as consecutive spawners, or wait until the spring of 2024, and instead spawn as skip spawners. The proportion of consecutive and skip spawners in a cohort varies annually and is detailed in Chapter 2, but in general, Yakima River fish predominately follow the consecutive spawner pathway (60-70%) and will spawn the following winter/spring, while the majority of Snake River kelts follow the skip spawner life history (60-70%), which has them held for an additional 12 months after capture, with subsequent spawning the next winter/spring. Fish strategy choice is likely controlled by a combination of genetics and environment.

Additional study is needed to evaluate reconditioning strategies for skip spawners. Our past approach was to hold the fish for an additional year in the hatchery and then release them. This scenario works well in the Snake River but is less successful in the Yakima River likely due to difficulties keeping intake water chilled adequately during the winter months. This is important, as the temperature regime of the spring water utilized at Prosser can be approximately 5-8 degrees warmer than ocean/river water during the winter months. This warmer water appears to cause maturation synchronization issues due to the prolonged holding that is needed for skip spawners. Also, the cost of holding fish for an additional year should be compared to results from other scenarios. We discontinued experimental releases from the Yakima River in 2022. We are currently evaluating any additional possible ways to improve skip spawner survival in the Yakima basin.

Summary Research-Scale Efforts to Address RPA 33 and subsequent Biological Opinion (2019)

At DNFH in 2022, we continued to conduct research toward addressing Reasonable and Prudent Alternative 33 for the Hydro-system Biological Opinion. The RPA 33 required the Action Agencies to develop, in cooperation with regional salmon managers, implementation of a Snake River steelhead kelt management plan, designed to provide at least a 6% improvement in B-run population productivity (NMFS 2008, 2010, and 2014). Toward that goal, a variety of approaches were tested and implemented including passage improvements and reconditioning kelt steelhead. The designation of B-run can be difficult to quantify in the Snake Basin. Historically, size has been used to determine B-run fish populations, which has been determined by genetic analysis to not be the sole determinant of B-run populations, since genetic assignments have provided data that B-run populations do not meet specific size requirements or overlap with A-run populations. To accurately measure how kelts contributed towards the 6% RPA 33 rule NOAA, CRITFC, Nez Perce Tribe, and the Federal Action Agencies (Bonneville Power Administration, U.S. Army Corps of Engineers, and the Bureau of Reclamation) devised a system that would recognize kelt reconditioning actions and give credit for those fish we successfully reconditioned and released towards the 6% RPA value (Kelt Master Plan Document) .

In the spring of 2019, the NOAA published a new Hydrological Biological Opinion for the Columbia River Power System (NMFS 2019). The new rules effectively retired the 6% number in 2019 and considered all future Snake River reconditioning actions as the only hatchery actions appropriate for corrective mitigation for steelhead loss in the Snake River. We have continued to maintain this evaluation to gauge how much progress we are making reconditioning kelts in the Snake River Basin.

Results/Discussion

Steelhead Kelt Collections

Large numbers of kelt steelhead are available for collection at many sites across the Columbia River Basin. These sites generally are associated with juvenile bypass systems or weirs. From 2002-2021 a total of 23,036 downstream migrating kelts at LGD were collected. Starting in 2020, a total of 97 kelts have been collected from Little Goose Dam, and 15,384 from the CJMF, from 2000-2020, which were collected and then either released back to the river or retained for reconditioning. The Columbia River, upriver steelhead run, in 2022-23, was an improvement but is still low in relation to previous recorded years since unclipped fish have been counted (FPC Data). We collected 111, 46, 25, 1, and 119 kelts at LGD, LGS, LMO, Asotin Creek, and CJMF, respectively (Table 1.1 and Appendix A). In 2022, the kelt collection represented 1.02 % and 21.6% of the upstream run in the Snake and Yakima rivers, respectively.

Reconditioning

Snake River collections since 2011, have retained 2,050 kelt steelhead for reconditioning, of these, 810 fish have survived to the following fall. Since 2000, 11,337 kelt steelhead were retained for reconditioning from collections at CJMF and 5,154 fish survived to that fall of the annual collection period (Appendix A). Snake River collections were made at the LGRJBF, starting in 2020 at LGSJBF, and at the LMOJBF in 2022, however it should be noted that kelt collections in the Snake River Basin have also come from the South Fork Clearwater River and Fish Creek (a Lochsa River tributary) in previous years (Hatch et al. 2018) and Asotin Creek in 2022.

Table 1. 1. Summary of kelt collections and fish disposition in 2022 from the Snake and Yakima Rivers.

	Lower Granite	Little Goose	Lower Monumental	Asotin Creek	Snake River total	Yakima River total
Total Collection	111	46	25	1	183	119
# Mature reconditioned fish released (consecutive spawners)	10	-	1	-	11	49
# Immature reconditioned fish retained (skip spawners)	45	10	2	0	57	0
# Immature reconditioned fish released (skip spawners)	NA	NA	NA	NA	NA	25
# Skip spawners released from previous collection	10	1	-	-	11	NA

Long-term reconditioning survival (from collection to the release in the fall) is variable from year to year but has averaged 45% at the Prosser Fish Hatchery (PFH) over the last 23 years. For the last 6 years, Yakima reconditioning has been 57.7% survival (Figure 1.1). The staff here have several years of reconditioning experience, so we generally observe only small annual variations in survival (Figure 1.1). The reconditioning survival rate for wild Snake River kelts from 2011 through 2022 is 39.5%. Survival during the initial years at DNFH (2011-12) was compromised as a result of poor water quality which has been detailed in previous reports (Hatch et al. 2012 and Hatch et al. 2013a) and compounded by the inexperience of new hatchery staff. However, the past 5 years have seen marked improvement in survival rates, as water quality issues have been improved and staff have gained additional experience in handling and caring for wild steelhead. The survival rate for the last five-year period combined at Dworshak National Fish Hatchery and Nez Perce Tribal Fish Hatchery have averaged 48.9% (Figure 1.2). In 2022 collections have increased to 183 collected, and long-term survival has been good (43%). Prevalence of the skip spawner life history continues to be much higher in the Snake River than in the Yakima River. On average, skip spawners compose just over 30% of the kelt collection in the Snake River, and approximately just over 20% in the Yakima River. This difference is likely the result of energy demands of a longer migration distance in the Snake River (Keefer et al. 2008). Figures (1.1) and (1.2) show differences in the life history strategy by the proportion of retained fish (skip spawners) at the two facilities. Reconditioned steelhead kelt releases for the Yakima River have been at historic lows due to poor river migration conditions (high temperatures and low flows), low ocean adult returns, and high water/flooding during collection periods. The improvement in Snake River releases have trended upwards in large part to successful retention and reconditioning of skip spawners (Figures 1.1 and 1.2) at the DNFH due to improved water quality, implementation of rearing protocols, improved life support systems, improved staff training, and experience.

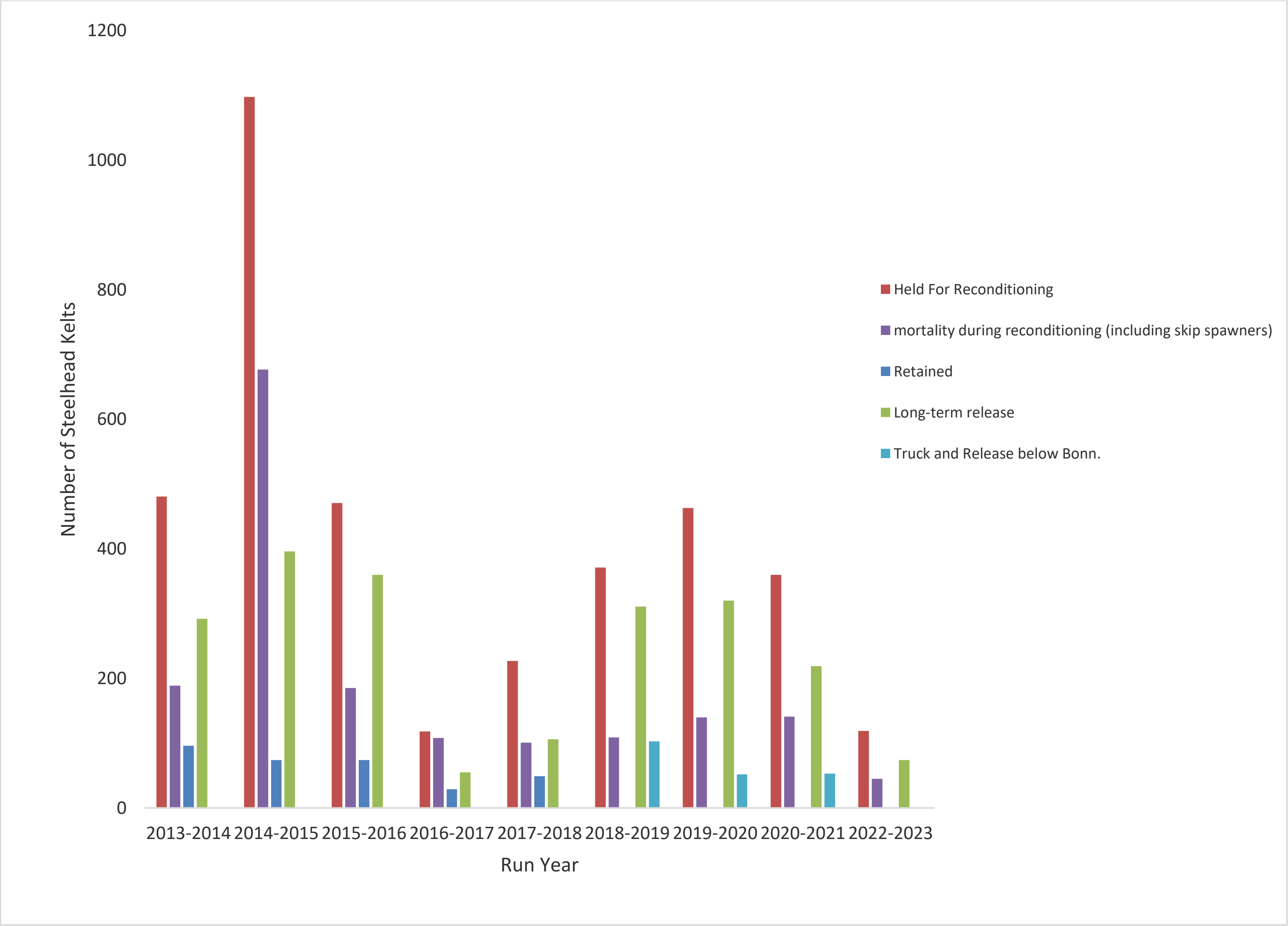


Figure 1. 1. Yakima River steelhead kelt collection for reconditioning and fate from 2013-2022. Beginning in 2019, kelts that would have been retained, were instead trucked, and released below Bonneville Dam, this was discontinued in 2022.

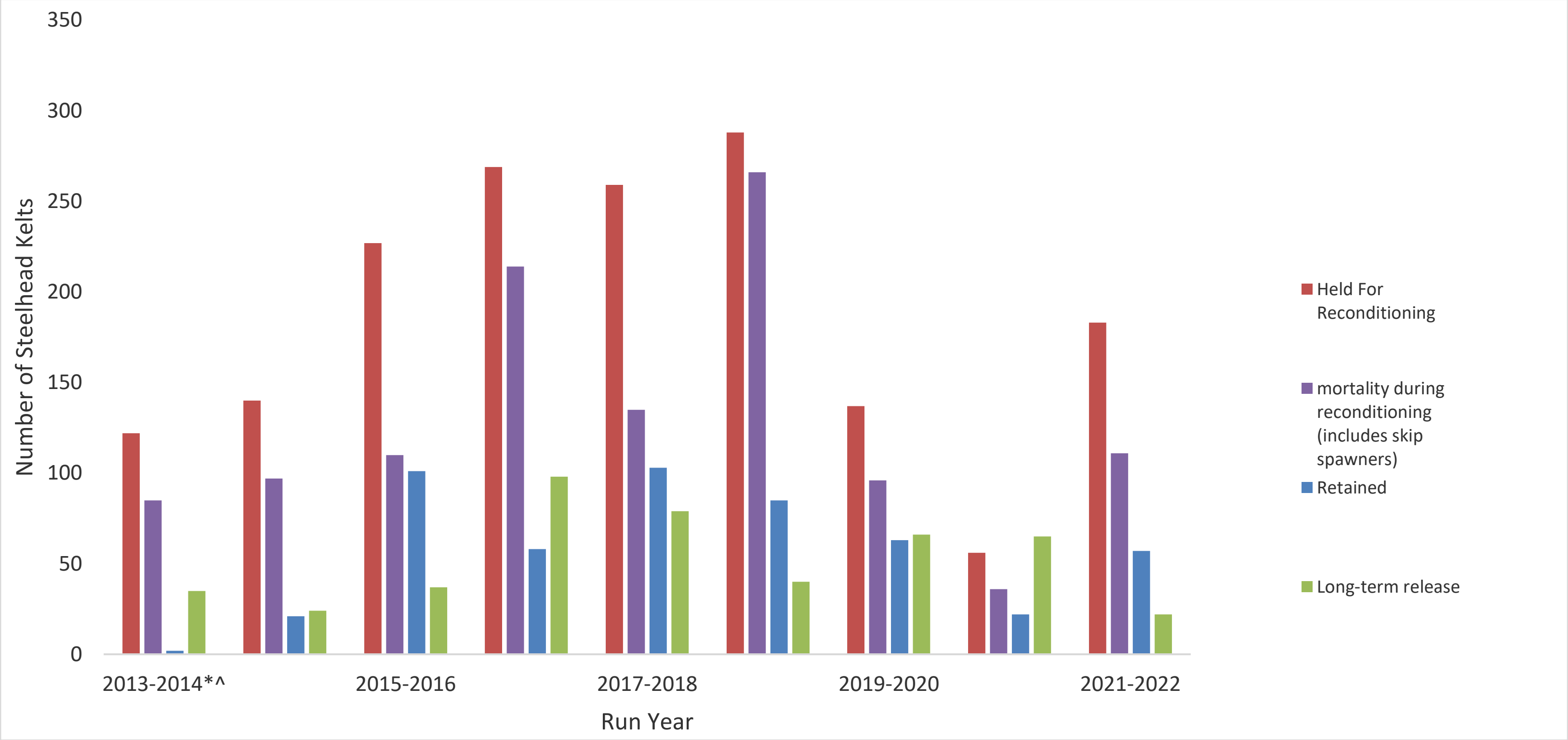


Figure 1. 2. Snake River steelhead kelt collection for reconditioning and fate from 2013-2022.

Skip Spawner Management Strategy in the Yakima River

Reconditioned kelt steelhead demonstrate either consecutive- or skip-spawning life histories. Skip spawners make up approximately 40% of collection in the Yakima River and 60% of the collection in the Snake River. Our primary management strategy for skip spawners was to hold the fish in the reconditioning for an additional year, thus releasing the fish in the second fall of captivity. This strategy has been successful in the Snake River where second year survival and rematuration of skip spawners has been very high (67.8% survival and 31.7% rematuration). In the Yakima River, this management strategy of holding skip spawners for a second year has been much less successful in terms of skip spawner survival. This likely is a result of warmer water temperature that is used to rear skip spawners during the winter. The water source is from a well that is ideal for summertime rearing, but that same water is much warmer than the average ambient river/ocean water temperatures where kelts would naturally recondition. This water temperature fluctuation likely had an impact on held skip spawner maturation timing.

Beginning in 2019, we experimented with transporting skip spawner kelts to the lower Columbia River and releasing them in the fall, concurrent with consecutive spawner releases in the Yakima River. This release strategy was discontinued in 2022 due to the lack of returns. We will be exploring different ways to improve skip spawner survival in the basin.

2019 Yakima River Lower Columbia River Release

We trucked and released 103 PIT-tagged, immature, reconditioned kelts below Bonneville Dam on 10/31/2019 to evaluate this management strategy. Thirty fish were detected moving upstream at the Bonneville Dam fish ladders within the first 30 days post release. Ten of these 30 fish had additional detections, four moving downstream, and six moving upstream. The four downstream moving fish were detected either in the Bonneville Dam juvenile bypass or corner collector. These migration patterns were consistent with post-spawn steelhead outmigration return to the ocean.

Final detection history for the six upstream moving fish includes three fish last heard at mainstem dams (2 at The Dalles and 1 at John Day ladders), and three fish last detected in the Yakima River tributaries. Tributary detections included one fish in Satus Creek. This fish genotyped to the Yakima River GSI reporting group and was likely a male based on genetic analysis. Another fish was last detected in Little Sheep Creek, a tributary of the Imnaha River in the Snake River basin. This fish genotyped to the MGILCS (mid-Columbia, Grande Ronde, Imnaha, lower Snake, lower Salmon, lower Clearwater) reporting group was likely a male based on genetic analysis (Hess et al. 2020). Finally, one fish was detected in Fifteen Mile Creek, located just upriver of the Dalles Dam, a small tributary off the mainstem Columbia River. This fish genotyped to the MGILCS reporting group and was likely male based on genetic analysis.

We will continue to monitor PTAGIS for possible detections from these lower river release groups. Skip spawning adult returns from the 2019 release group would be expected back in the summer/fall of 2021, but based on PTAGIS detections there were no returns from this group in 2021 or 2022.

2020 Yakima River Lower Columbia River Release

On October 21, 2020, we released 52 PIT-tagged, immature, reconditioned kelts below Bonneville Dam. Due to COVID-19 concerns, blood draws were not conducted at Prosser to determine maturation status. Instead, visual maturation was determined by the hatchery manager. We initially observed 8 of these kelts moving upstream at Bonneville Dam. One fish has migrated back to Prosser already, which was a female, based on earlier visual calls when selected for reconditioning. Likely, this fish maturation status was called incorrectly and will be a consecutive spawner. We anticipate a that more thorough analysis will be ready in 2021, which will include genetic analysis to accurately determine sex and GSI origin.

We continue to monitor PTAGIS for any possible return detections which we would anticipate beginning as early as July of 2021, possibly going into 2022. At the date of this report, we have had no detections of these fish returning from the ocean.

2021 Yakima River Lower Columbia River Release

On October 28, 2021, we released 53 PIT-tagged, immature, reconditioned kelts below Bonneville Dam. Blood draws were resumed in 2022, and these fish were determined to be immature based on the blood hormone profiles established in the lab. At the date of this report, we have had no detections of these fish returning from the ocean.

Long-term reconditioning mature kelt release

We evaluated the traits and survival-to-release of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River (Hatch et al. 2013b). Reconditioned steelhead kelts continue to be predominantly (>92%) female. The annual survival to release average ranged from 18% at the start of the program in the early 2000's to an annual high of 76% in 2016 and averaged 45% over the course of the study (2000-22) with surviving reconditioned kelts showing increases in fork length, weight, and Fulton's K condition factor. Kelts in good condition, and those with bright coloration at the time of collection were more likely to survive. Post-release upstream migration timing of reconditioned kelts was spread out over several months and correlated well with run timing of upstream pre-spawn migrants. The empirical results we observed demonstrate the potential of kelt reconditioning to provide recovery benefits for ESA-listed, repeat spawning steelhead populations in highly developed river systems. See Appendix A for annual data.

In Figures 1.3 and 1.4, the numbers of female kelts reconditioned and released are added to the corresponding steelhead run. For example, a consecutive kelt that is collected in the Spring of 2014 (from the 2013/2014 run) would be released into the following run year of 014/2015. For a skip spawner, which is typical of Snake River kelt, a fish caught in the Spring of 2014 would be released into the 2015/2016 run year. Figures 1.3 and 1.4 demonstrate that the contribution of reconditioned kelts to the overall runs in the Yakima and Snake rivers is measurable and quite substantial in comparison to no intervention. While the total number of returned fish may appear small compared to the overall run, especially in the Snake, many of these fish are contributing towards ESA-listed populations throughout the Yakima and Snake basins. In Figure 1.4, both Snake River A and B-run populations are counted. We primarily targeted B-run fish while selecting kelts for reconditioning although there were a small proportion of A-run fish that were also collected and reconditioned. The A-run population in the Snake is much larger than the B-run population, with most of these fish coming from the Grande Ronde basin (Hatch et al. 2019).

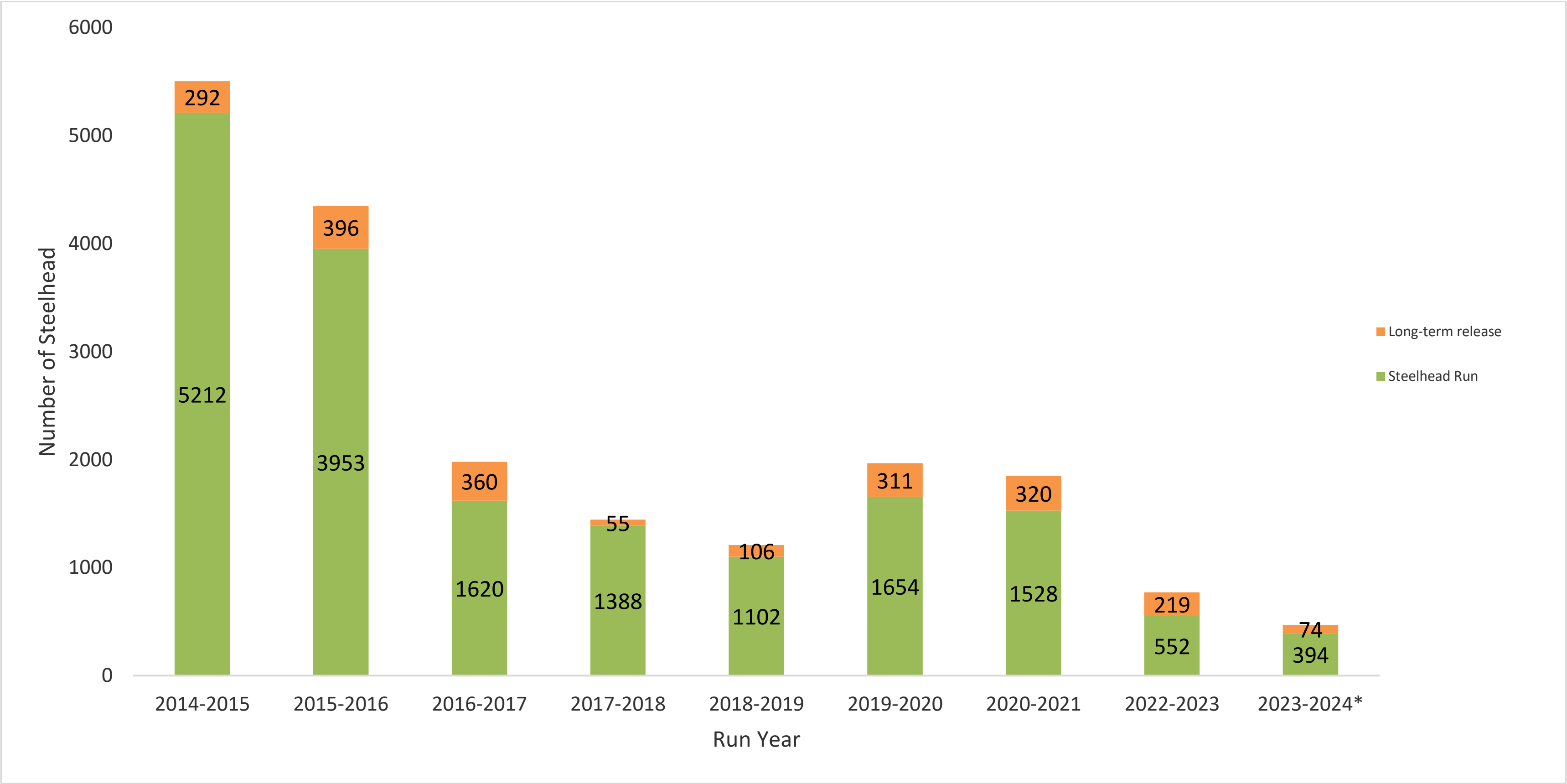


*Figure 1. 3. Contribution to steelhead run from reconditioned kelt release in Yakima Basin. * Run is still ongoing; value will be updated in 2023 steelhead kelt reconditioning annual report.*

*Figure 1. 4. Contribution to steelhead run from reconditioned kelt release in Snake Basin at LGD. * Includes Fish Creek and/or South Fork Clearwater Kelts. Data label marked with x is preliminary, will be updated in 2023 annual report.*

The natural repeat spawner rate (or no intervention) measured as a return rate to Bonneville Dam, for the Snake River, is 0.27% and 2.90% for the Yakima River. The calculated benefit of reconditioning relative to leaving the fish in the river is 164.7 times for the Snake River and 15.4 for the Yakima River.

Summary Research-Scale Efforts to Address RPA 33 and subsequent Biological Opinion (2019)

Since operating at a research scale in the Snake Basin, as approved by the ISRP in the 2008 review, the capacity of our facility was much too small to meet the RPA 33 goal of increasing the LGR ladder count of B-run steelhead by 6%. However, we have demonstrated the feasibility of reaching the 6% goal. Releases of successfully reconditioned mature kelts began in 2011, but due to water quality issues in both 2011 and 2012 (Hatch et al. 2012 and 2013) these numbers were severely under representative of what we could accomplish at the initial experimental site (Dworshak National Fish Hatchery) and with the inclusion of an additional temporary reconditioning site in 2016 (Nez Perce Tribal Hatchery). In 2020 through 2022, we only reared fish at DNFH due to low collection numbers. Table (1.1) summarizes all collections for both A and B-run, and releases associated with the RPA 33.

In 2013, we had a successful reconditioning and release at nearly 40% towards reaching the RPA 33 goal with 69 female fish released (Table 1.2). Our best year was in 2017, with releases just over 50% towards the RPA goal, at 98 mature female spawners released. In 2019, we had a lower number of mature kelts to release due to a mishap at the Nez Perce Tribal Hatchery, which resulted in a significant loss of retained skip spawner kelts from 2018. Nez Perce Tribe is taking actions to prevent such a catastrophe in the future, with protocols that should help to eliminate the chance that such an event will happen again. Since 2013, we have averaged 32% of the RPA goal, releasing an average of 56 mature female fish per year with a total of 525 mature female fish released from 2011-2022 (Table 1.2). Figure 1.5 is the schema that was devised by the Power Agencies and CRITFC to determine the credit that would be allocated towards collection and successful reconditioning of what was considered B-run kelt steelhead towards the larger B-run the year of release. The number represents the B-run steelhead released x estimated successful reproductive contribution. This is not representative of number of total kelts released and was generated under the direction of RPA 33. We will continue to estimate B-run steelhead kelt contributions in future reports for the benefit of management implications of kelt reconditioning in the Snake River Basin.

Table 1. 2. Summary of fish collections and releases in the Snake River associated with RPA 33.

Year	**Collection Location**	**Number of Fish Collected**	**Number of Fish that Survived Reconditioning**	**% Survival**	**Consecutive Spawner Release**	**Number of Fish Retained**	**Mature Skip Spawners Released (Capture Year)**	**Total Release by Year**
2011	Lower Granite Dam	111	2	1.8%	2	-	-	-
2011	S.F. Clearwater	-	-	-	-	-	-	-
2011	Fish Creek	-	-	-	-	-	-	-
2011 (subtotal)		**111**	**2**	**1.8%**	**2**	**-**	**-**	**2**
2012	Lower Granite Dam	124	10	8.1%	10	-	-	-
2012	S.F. Clearwater	-	-	-	-	-	-	-
2012	Fish Creek	-	-	-	-	-	-	-
								-
2012 (subtotal)		**124**	**10**	**8.1%**	**10**	**-**	**-**	**10**
2013	Lower Granite Dam	110	57	51.8%	57	-	-	-
2013	S.F. Clearwater	24	12	50.0%	12	-	-	-
2013	Fish Creek	-	-	-	-	-	-	-
2013 (subtotal)		**134**	**69**	**51.5%**	**69**	**-**	**-**	**69**
2014	Lower Granite Dam	110	34	30.9%	34	-	-	-
2014	S.F. Clearwater	-	-	-	-	-	-	-
2014	Fish Creek	12	3	25.0%	1	2	2	-

2014 (subtotal)		**122**	**37**	**30.3%**	**35**	**2**	**2**	**35**
2015	Lower Granite Dam	22	11	50.0%	8	3	3	8
2015	S.F. Clearwater	35	7	20.0%	4	3	0	4
2015	Fish Creek	83	25	30.1%	10	15	15	12*
2015 (subtotal)		**140**	**43**	**30.7%**	**22**	**21**	**18**	**24**
2016 (subtotal)	Lower Granite Dam	**227**	**120**	**52.9%**	**19**	**101**	**77**	**37*^**
2017 Subtotal	Lower Granite Dam	**269**	**59**	**21.9%**	**21**	**58**	**29**	**98^**
2018 Subtotal	Lower Granite Dam	**259**	**177**	**68.3%**	**50**	**99**	**1**	**79^**
2019 Subtotal	Lower Granite Dam	**288**	**121**	**42.0%**	**39**	**85**	**58**	**40^**
2020	Lower Granite Dam	88	53	60.2%	7	46	58	65^
2020	Little Goose Dam	49	18	36.8%	1	17	3	1
2020 Subtotal		**137**	**71**	**51.8%**	**8**	**63**	**61**	**66^**
2021	Lower Granite Dam	44	21	47.7%	4	17	10	4
2021	Little Goose Dam	12	1	8.3%	0	1	1	0
2021 Subtotal		**56**	**22**	**39.3%**	**4**	**18**	**11**	**65^**

2022	Lower Granite Dam	111	66	**59.5%**	**10**	**20**	**TBD 2023**	**10**
2022	Little Goose Dam	46	10	**21.7%**	**0**	**1**	**TBD 2023**	**0**
2022	Lower Monumental Dam	25	3	**12.0%**	**1**	**1**	**TBD 2023**	**1**
2022	Asotin Creek	1	0	**0.0%**	**0**	**0**	**0**	**0**
2022 Subtotal		**183**	**79**	**43.2%**	**11**	**22**	**TBD 2023**	**22^**
Grand Total		**2050**	**810**	**39.5%**	**290**	**549**	**257**	**547**
				***Includes Fish Cr. kelt skip spawners.**				
				^Includes previous year kelt spawners from LGD and LGO.				

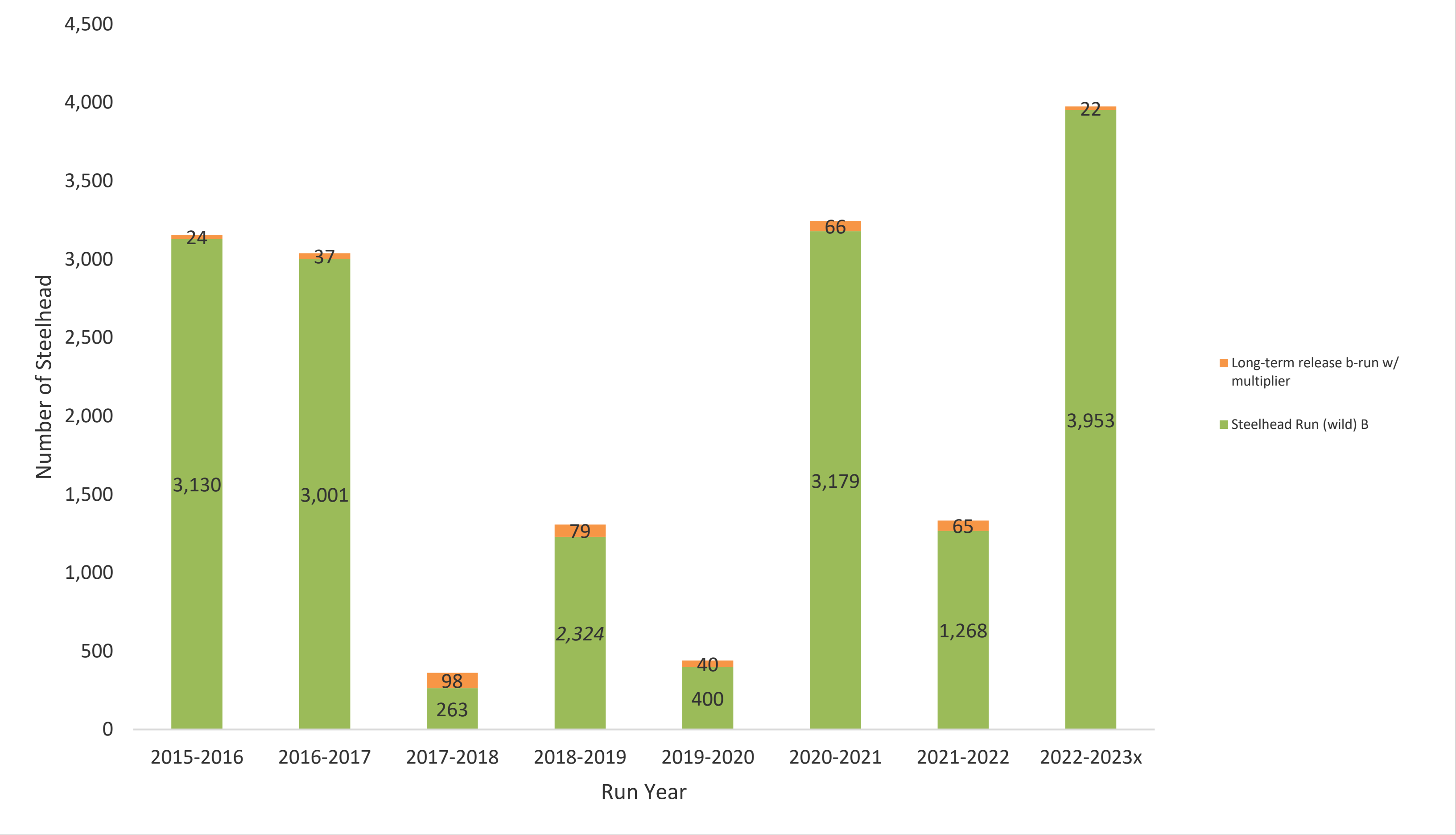


Figure 1. 5: Contribution to steelhead run from reconditioned kelt release in Snake River Basin. x2022-2023 value is an estimated run size and will be corrected in the 2023 Kelt Annual Report. B-run numbers utilize Kelt Master Plan definition for reconditioned female kelt spawners

Chapter 2. Cumulative Results

This section was part of our response to the Northwest Power and Conservation Council's recent Anadromous Habitat and Hatchery Projects Project Review (ISRP 21-8). It's included to provide a comprehensive presentation of project results over the last decade.

Steelhead kelt reconditioning programs collect steelhead kelts (post-spawned adult steelhead) to recondition them so that they can be released into the river to spawn again. Steelhead kelt reconditioning is the practice of capturing, holding, and feeding post-spawned steelhead in an artificial rearing environment for the purpose of regeneration of gonads for repeat spawning (circumventing the migration down the river, ocean rearing, and migration back to tributaries). Reconditioned kelts are released back into the river after 6 or 18 months depending on the rematuration schedule.

Starting in 2000, CRITFC and the YN collaborated to investigate steelhead kelt reconditioning. At the time, little was known or published about kelt steelhead reconditioning. Consequentially, initial work focused on very basic components: collecting kelts, how to do basic fish care, feed types and rations, treatments, evaluating maturation status, release strategies, and other basic components. We used literature published on Atlantic salmon kelt reconditioning as a starting point, but a lot was developed from trial, error, and adaptive management. Early reviews from the ISRP helped define areas for research and investigation, including evaluating the relative reproductive success of reconditioned kelt steelhead, as well as developing an understanding of kelt physiology. The Columbia Basin Fish Accords were signed in 2007 and at the same time we were seeing encouraging results from the Yakima River Kelt Project, so we expanded to include a Snake River component and a collaboration with the Nez Perce Tribe. The ISRP posed five questions to a sister Yakama Nation project (2008-458-00) in a 2014 review:

1. "The prior recommendation, by the ISRP, to establish methods to assess how kelt reconditioning may benefit population growth, abundance, spatial structure, and diversity still needs to be addressed.
2. Some modeling and a power analysis need to be conducted to clarify how many juveniles and F1 adults should be sampled to detect meaningful differences in the breeding and reproductive success of hatchery-origin, natural-origin, and reconditioned natural-origin females.
3. Methods to assess the fat levels, maturation timing, fecundity, egg size, and gamete viability of the project's reconditioned kelts need to be developed and implemented. The fate of non-maturing or skip-spawning reconditioned fish should be documented.
4. Viable plans are needed to monitor the homing and straying rates of reconditioned kelts released by the project.
5. Experiments are needed to discover the best geographic locations and times of year for release of the project's reconditioned fish."

We have used these questions as guidance for our studies since 2014 and we will organize our response on progress to date around these themes. This project has published 18papers on these subjects since 2013. The newest one is located in the Appendix B.

What are the benefits to the receiving population in terms of Viable Salmonid Population (VSP) parameters?

Our project includes both Yakima and Snake rivers components and information will be provided on each of these systems. Primary focus will be on the Yakima River to build the case of benefits to population growth, abundance, spatial structure, and diversity due to differences in scale between the two systems.

From 2001 to 2022, a total of 11,337 out-migrating kelts were collected at the Chandler Juvenile Collection Facility (Table 2.1) and held for reconditioning. All captured fish were PIT tagged for individual identification during reconditioning and following release. Captured kelts were held at the Prosser Hatchery for reconditioning. The average survival rate of reconditioned kelts to release was 45%. On average, 493 kelts were collected and 220 were released annually at this location from 2001-2022 (Table 2.1). The majority (>92%) of kelts were female (Hatch et al. 2019). We evaluated the traits and survival to release of reconditioned kelt steelhead in the Yakima River program from 2001 to 2011 in an accredited peer reviewed publication (Hatch et al. 2013b).

Table 2. 1. Yakima Basin steelhead kelts reconditioned at Prosser Hatchery, number of kelts released, and survival rate to release, 2001 to 2022. Source: Yakama Nation.

Year	Number of Kelts Collected	Number of Reconditioned Kelts Released	Survival to Release
2001	508	108	21.3%
2002	420	142	33.8%
2003	482	301	62.4%
2004	694	288	41.5%
2005	427	86	20.1%
2006	279	85	30.5%
2007	422	221	52.4%
2008	472	266	56.4%
2009	510	141	27.6%
2010	1,100	426	38.7%
2011	680	223	32.8%
2012	572	333	58.2%
2013	575	308	53.6%
2014	481	316	74.4%
2015	1,098	435	43.4%
2016	525	341	73.0%
2017	117	84	71.8%
2018	227	152	67.0%
2019	371	248	66.9%
2020	463	320	69.1%
2021	360	219	60.8%
2022	119	74	62.2
Total	*11337*	*5063*	*44.7% (pooled)*
Average	*493*	*220*	*46.7%*

Reconditioned kelts were released adjacently upstream of Prosser Dam from 2001 to 2007, and from 2008 to present, approximately one kilometer downstream of Prosser Dam. Reconditioned kelts are released into the river from mid-October to early December (when water temperature of rearing water and the river were in conjunction) when the majority of the natural run is returning from the ocean. Reconditioned fish mingle with the natural run, then proceed to overwintering locations to hold, and then proceed on to spawning grounds in the spring to create redds and mate. The release location was selected to allow reconditioned kelts to migrate upstream and choose their spawning location volitionally. Spawning in the Yakima River basin generally peaks from early March to early May depending on the elevation of the stream (goes from Satus, Toppenish, Ahtanum, Naches, and upper Yakima). The timing of upstream migration by reconditioned kelts from 2008-present was determined based on PIT tag detections at Prosser Dam.

We compared return rates of repeat spawners among long-term reconditioning, short-term reconditioning, immediate transport to the lower Columbia River, and control groups in the Yakima River. Long-term reconditioning demonstrated significantly higher return rates of repeat spawners (11-18%) than other treatments (1-3%) (Table 2.2). This result was supported despite the typical variation in river, ocean, and fish conditions among the years that were incorporated into the error term in our analysis. The data extrapolation required in our analysis does not account for variation in environmental or fish conditions between years. However, this method does provide a best and worst-case interpolation of data for earlier years in the long-term reconditioned group, thereby strengthening our ability to draw conclusions among the four treatments. For more in-depth analysis see our publication (Trammell et al. 2016).

Table 2. 2. Sample size (N), mean, and grouping output for Tukey post-hoc test from ANOVA of PIT tag detections at Prosser Dam. From Trammell et al. 2016.

Treatment	**N**	**Mean**	**Grouping**
Long-term min	10	11.5	A
Long-term max	10	17.6	A
Short-term	7	3.2	B
Transport	7	0.9	B
Control	7	2.7	B

Table (2.3) gives the count of upstream migrating ocean-returning steelhead, reconditioned kelts released that were subsequently detected migrating upstream, and the combined total steelhead run year count at Prosser Dam for the most recent 14 return years. The percentage of the total return that consisted of actively migrating reconditioned kelts averaged just over 5.0% over these 14 years. Since we know reconditioned kelts are predominantly female, these data demonstrate a substantial and measurable contribution to overall population demographics in comparison to no intervention. Based on average survival to release (~50%; Table 2.1) and release to spawning ground migration (~50%; Table 2.3), approximately 25% of kelts collected for reconditioning are expected to migrate to the spawning grounds.

Table 2. 3. Contribution to steelhead spawning run from reconditioned kelts released in the Yakima basin.

Run Year	Ocean Return	Reconditioned Kelts[1]	Total	Reconditioned Kelts
2008-09	3,378	91	3,469	2.6%
2009-10	6,750	46	6,796	0.7%
2010-11	6,043	153	6,196	2.5%
2011-12	6,301	58	6,359	0.9%
2012-13	4,556	231	4,787	4.8%
2013-14	4,039	104	4,143	2.5%
2014-15	5,096	116	5,212	2.2%
2015-16	3,737	216	3,953	5.5%
2016-17	1,462	155	1,617	9.6%
2017-18	1,335	53	1,388	3.8%
2018-19	998	95	1,093	8.7%

2019-20	1,542	115	1,657	6.9%
2020-21	1,385	143	1,528	9.4%
2021-22	657	97	754	12.9%
2022-23	390*	4*	394*	TBD 2023

1 Kelts detected migrating upstream (natural and hatchery-origin). * Run is still ongoing at the time of this report.

Relative to other populations, adult productivity indices for Yakima River MPG steelhead are presently trending upward and Figure (2.1) indicates that Yakima River MPG steelhead are experiencing higher survival relative to other steelhead populations above Bonneville Dam. This could be a positive result of the kelt reconditioning program, though separating effects of habitat improvement projects and kelt reconditioning is difficult to determine.

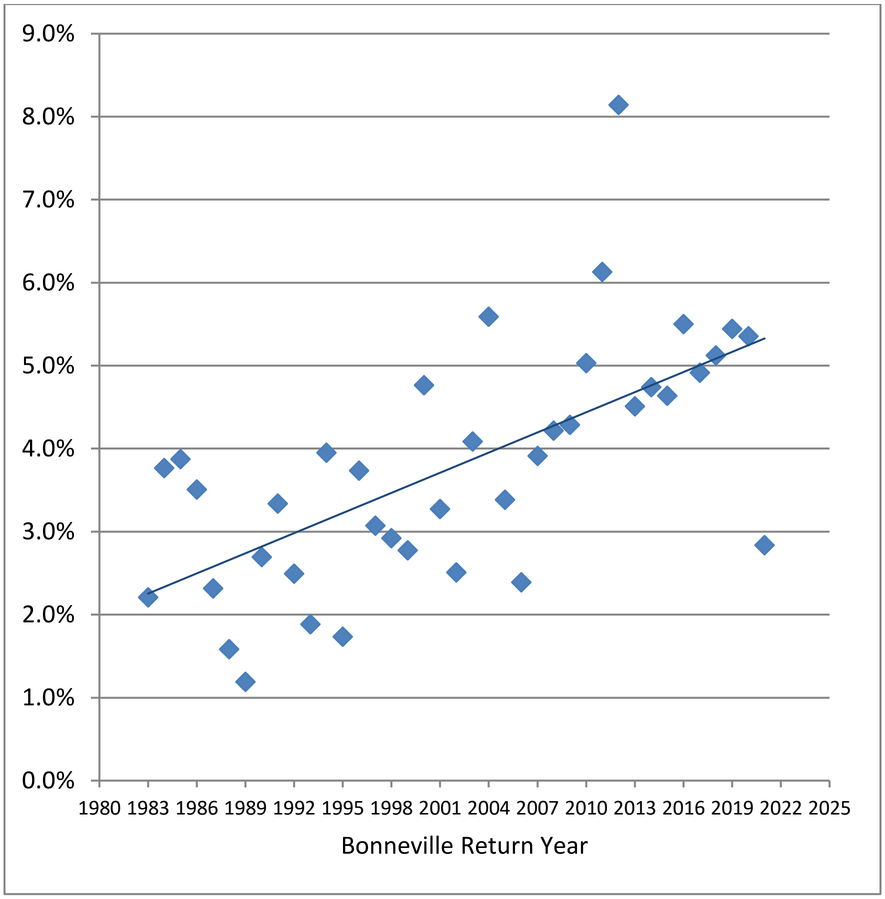


Figure 2. 1. Yakima River MPG steelhead (Prosser wild abundance) as a percentage of Bonneville Dam wild Group A steelhead abundance, 1983 to 2021.

Impacts to spatial structure can be seen in data from the Snake River. We collect kelt steelhead at Lower Granite and recently at Little Goose and Lower Monumental dams, so the collection is a mixture of nearly all Snake River populations. As abundance of wild fish has decreased, our collection criteria for fish length has decreased as well, so as to spread benefits to all populations, not just to the B-run component. All reconditioned kelts are PIT tagged and released downstream of Lower Granite Dam in the fall. Figure 2.2 and Table 2.4 displays the point of last PIT detection, after Lower Granite Dam Adult ladder, for reconditioned kelts released in 2017, 2018, and 2019. Below the figure is a key to the PIT array locations and the number of different fish with final detections at each site. Though this is a coarse look at distribution, it does show that reconditioned fish are traveling throughout the Snake River Basin to find spawning grounds. There are still many tributaries within the Snake River Basin that do not have a PIT antenna array installed in them.

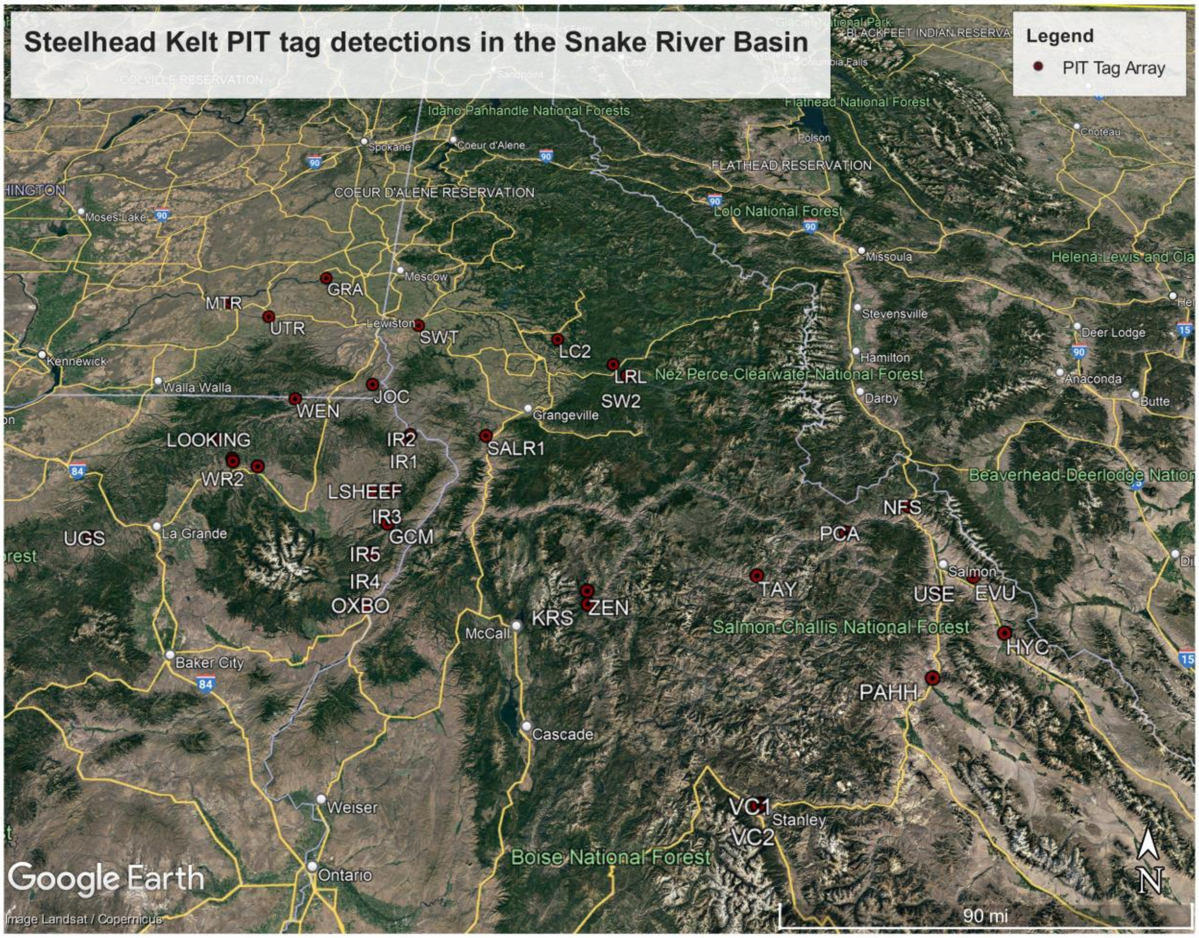


Figure 2. 2. Map of the Snake River basin with yellow pins indicating the last PIT detection location for reconditioned steelhead released in 2017-2022. Below is the key to PIT sites and the tally of fish with final detection at each site.

Table 2. 4. Corresponding sites by year, detailed location information, and number of detections by individual fish. ** 2022 data is preliminary as fish are still migrating at the time of this report.*

SITE	SITE DETAILS	2017	2018	2019	2020	2021	2022
ACB	ASOTIN CREEK @ CLOVERLAND BRDG SNAKE		1				
AFC	NO/SO FORK ASOTIN CR SNAKE		1				
BHC					1		

BSC	BIG SHEEP CR @ KM 6 IMNAHA	2	1	1	3	2	
CCW	CATHERINE CREEK LADDER/WEIR GRANDE RONDE		2	1			
ESS	EFSF Salmon River at Parks Cr.				1		
EVU	Eagle Valley Ranch Upper					1	
GCM	Grouse Creek Mouth					2	
GRA	LOWER GRANITE DAM SNAKE	17	46	5	32	25	16*
HYC	Hayden Creek In-stream array				1	1	
IR1	LOWER IMNAHA RIVER @ KM 7 IMNAHA		2				
IR2	LOWER IMNAHA RIVER @ KM 10 IMNAHA		4		1	3	
IR3	UPPER IMNAHA @ KM 41 IMNAHA	2	5	3	1	1	
IR4	Imnaha Weir Downstream Array					1	
IR5	IMNAHA WEIR UPSTREAM IMNAHA		2			1	
JOC	JOSEPH CREEK @ KM 3 GRANDE RONDE	2	6	3	1	1	
KHS	BIG BEAR CREEK @ KENDRICK HS POTLATCH		1				
KRS	SF SALMON RIVER @ KRASSEL CR SALMON			1	1		
LAP	LAPWAI CREEK NEAR MOUTH CLEARWATER	1			1		
LC1	LOWER LOLO CREEK @ KM 21 CLEARWATER	1	1	1			
LC2	UPPER LOLO CREEK @ KM 25 CLEARWATER	2					
LOOKING	LOOKINGGLASS HATCHERY (PASSIVE) GRANDE RONDE		1				

LRL	LOWER LOCHSA RIVER LOCHSA			1	1		
LRU	LOCHSA RIVER UPPER SITE LOCHSA	5	2	1			
LSHEEF	Listtle Sheep Facility					3	
MR1	MINAM RIVER @ KM 0.5 WALLOWA			3	4		
MTR	Middle Tucannon River					1	
NFS	NF SALMON RIVER @ KM 0.5 SALMON		2				
OXBO	Oxbow Hatchery (IDFG)					1	
PAHH	Pahsimeroi Hatchery				2		
PCA	PANTHER CREEK SALMON		1			1	
SALR1	SALMON RIVER MOUTH TO KM 171 SALMON			1			
SC1	LOWER SF CLEARWATER @ KM 1 CLEARWATER		1				
SC2	LOWER SF CLEARWATER @ KM 2 CLEARWATER		1	1	1		
SWT	SWEETWATER CREEK NEAR MOUTH CLEARWATER		1		2		
SW2	Upper Selway River Array				1		
TAY	BIG CREEK @ TAYLOR RANCH SALMON		1			1	
UGR	UPPER GRANDE RONDE @ KM 155 GRANDE RONDE		2			2	
UGS	UPPER GRANDE RONDE STARKEY GRANDE RONDE	1	1		1	1	
USE	UPPER SALMON RIVER @ KM 437 SALMON		1	1		2	
USI	Upper Salmon River @ km 460					1	

UTR	UPPER TUCANNON RIVER @ KM 53.2 TUCANNON	1	1				
VC1	VALLEY CREEK UPSTREAM SITE SALMON	1					
VC2	VALLEY CREEK DOWNSTREAM SITE SALMON	1					
WEN	WENAHA RIVER MOUTH GRANDE RONDE		3		2		
WR1	WALLOWA RIVER @ KM 14 WALLOWA	1	1				
WR2	WALLOWA RIVER @ KM 32 WALLOWA			1	4	7	
ZEN	SECESH RIVER @ ZENA RANCH SALMON		2				
TOTAL		**36**	**93**	**24**	**61**	**58**	**TBD 2023**

Kelt reconditioning has a direct impact on population diversity by amplifying life history complexity (Jenkins 2018). Repeat spawner steelhead are very scarce in most interior Columbia Basin streams (Keefer et al 2008 and Keefer et al. 2016). Kelt reconditioning infuses consecutive and skip spawn life history variants into the receiving populations. Moore et al. (2014) provided evidence that life-history diversity can diminish variability in population abundance and biomass via portfolio effects. Kelt reconditioning can benefit the receiving population through simply increasing spawner abundance, but also because repeat spawners are larger and have the potential for greater individual productivity (Jenkins et al. 2018). Further, simulations reported by Moore et al. (2014) showed a relationship between the proportion of repeat spawners in the population and the stability and persistence of the steelhead population. Therefore, increasing repeat spawner abundance through kelt reconditioning may stabilize population dynamics through several pathways.

What is the reproductive success of reconditioned kelts?

Reconditioned kelts are able to spawn in the wild and have been shown to have relative fitness similar to that predicted and seen in a study of natural kelts (Seamons and Quinn 2010). We have focused our primary research on relative reproductive success in both Toppenish and Satus creek drainages where lifetime reproductive success of female reconditioned kelts has averaged 2.41 that of single spawn fish as measured across 8 consecutive spawn years. Toppenish and Satus creeks (Yakima River tributaries) were selected as good study locations after encountering difficult logistical issues in other locations. Post-spawn collections at the Chandler facility provides a reasonable number of fish to recondition. Reconditioned kelts are allowed to overwinter in the mainstem Yakima River prior to the second spawning event which allows for a volitional return and biological (physiological) synchronicity with the natural system. PIT tag arrays detect both first time spawner and reconditioned kelt migration events. Redd surveys allow us to identify areas likely to have high proportions of offspring from anadromous adults, and to minimize the collection of non-target resident fish. Availability of age-0 samples allows identification to brood year to differentiate between first and second spawning events.

Sample Collection

Juvenile collection and analysis were narrowed to Satus and Toppenish Creeks and their tributaries (Hatch et al. 2011). These systems have the best PIT-tag antenna stability, largest number of successfully collected and reconditioned kelts, access to sampling, and lowest impact to spawning habitat, which all translates into good sample sizes for analysis. The biggest downside at both locations is that during good run years, maiden fish comprised a much larger portion than the Prosser reconditioned kelts. Since 2013, sampling locations for juveniles are seen in Figure (2.3). Some sampling sites were not sampled across all years due to access constraints.

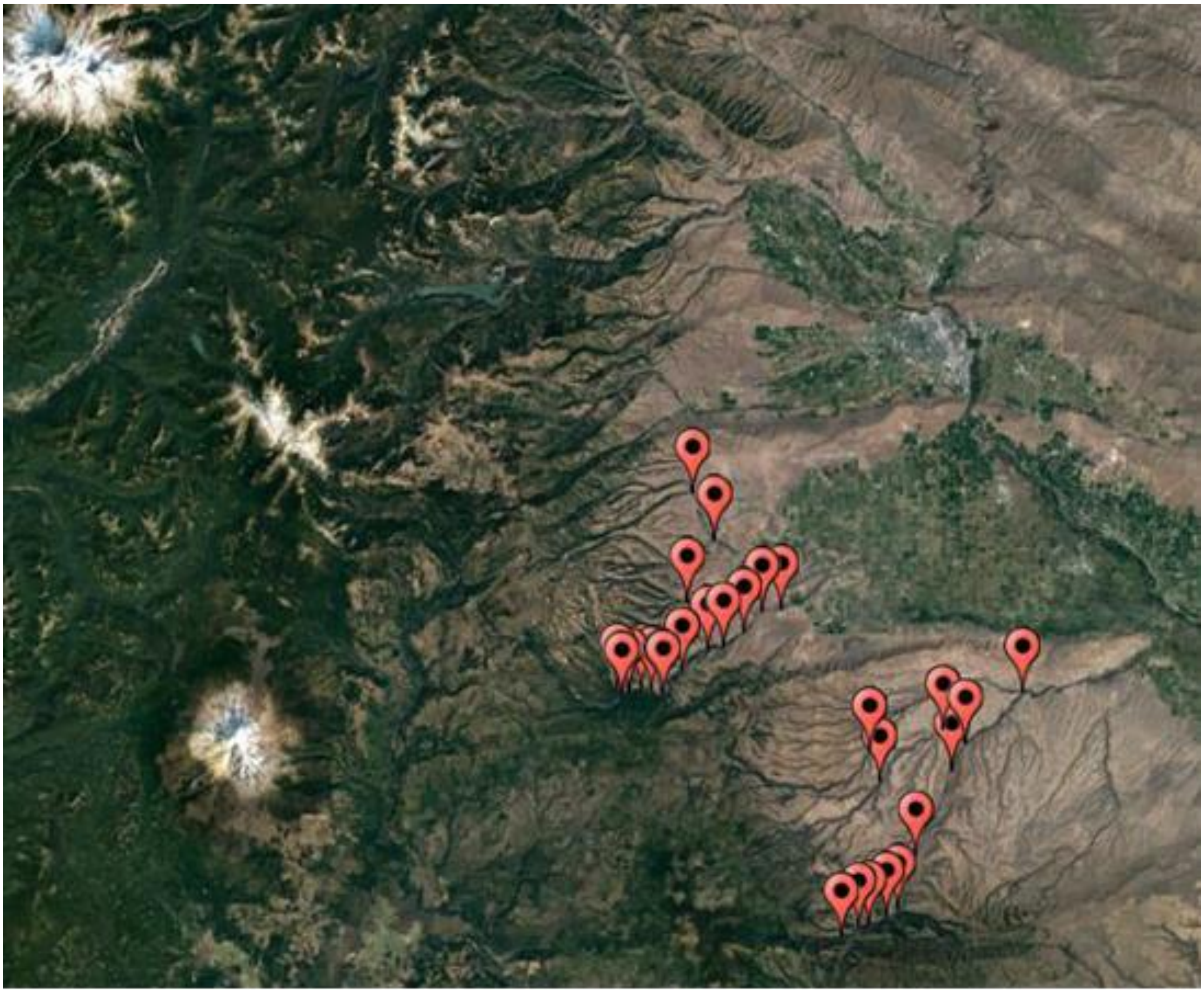


Figure 2. 3. Juvenile sampling locations in Satus (lower right) and Toppenish creeks over 8 years 2013-2021.

Results (2013-2021)

The number of juveniles successfully genotyped at the tributary level, and the corresponding number and percentage of samples assigned to at least one anadromous adult parent is shown in Table (2.5). This table does not include locations dropped because of access issues, low sampling success, or lack of assignments to an anadromous offspring. The low apparent assignment rate is due to the low number of adults and juveniles sampled compared to the relative number of unsampled anadromous steelhead and potential resident fish. In Figure (2.4), the largest number of adult fish have 0 progeny detected. This does not indicate that fish are not reproducing but that finding progeny in nature is a difficult proposition, sampling was limited, and missing assignments appears to be equally distributed amongst all groups. This holds true for fish that had a single progeny assignment as well. The second part of the Figure (2.4) has a smaller y-axis to better show the variation between spawning event types. Kelt spawning events appear to have a similar distribution of progeny when compared to maiden events (Figure 2.4).

Table 2. 5. Number of juveniles genotyped by tributary and subsequently assigned to at least one anadromous adult using parentage analysis. Results are reported for individual years and cumulatively across all years.

		Satus Cr.	Toppenish Cr.
2013	Genotyped	227	204
	Assigned	54	64
	% Assigned	24%	31%
2014	Genotyped	285	231
	Assigned	64	67
	% Assigned	22%	29%
2015	Genotyped	341	369
	Assigned	123	165
	% Assigned	36%	45%
2016	Genotyped	790	524
	Assigned	288	187
	% Assigned	36%	36%
2017	Genotyped	442	578
	Assigned	136	172
	% Assigned	31%	30%
2018	Genotyped	615	589
	Assigned	171	165
	% Assigned	28%	28%
2019	Genotyped	651	592
	Assigned	299	239
	% Assigned	46%	40%
2020	Genotyped	630	439
	Assigned	172	77
	% Assigned	27%	18%
2021	Genotyped	1349	668
	Assigned	541	237

	% Assigned	40%	35%
Sum	Genotyped	4,649	4,194
	Assigned	1,549	1,373
	% Assigned	33%	33%

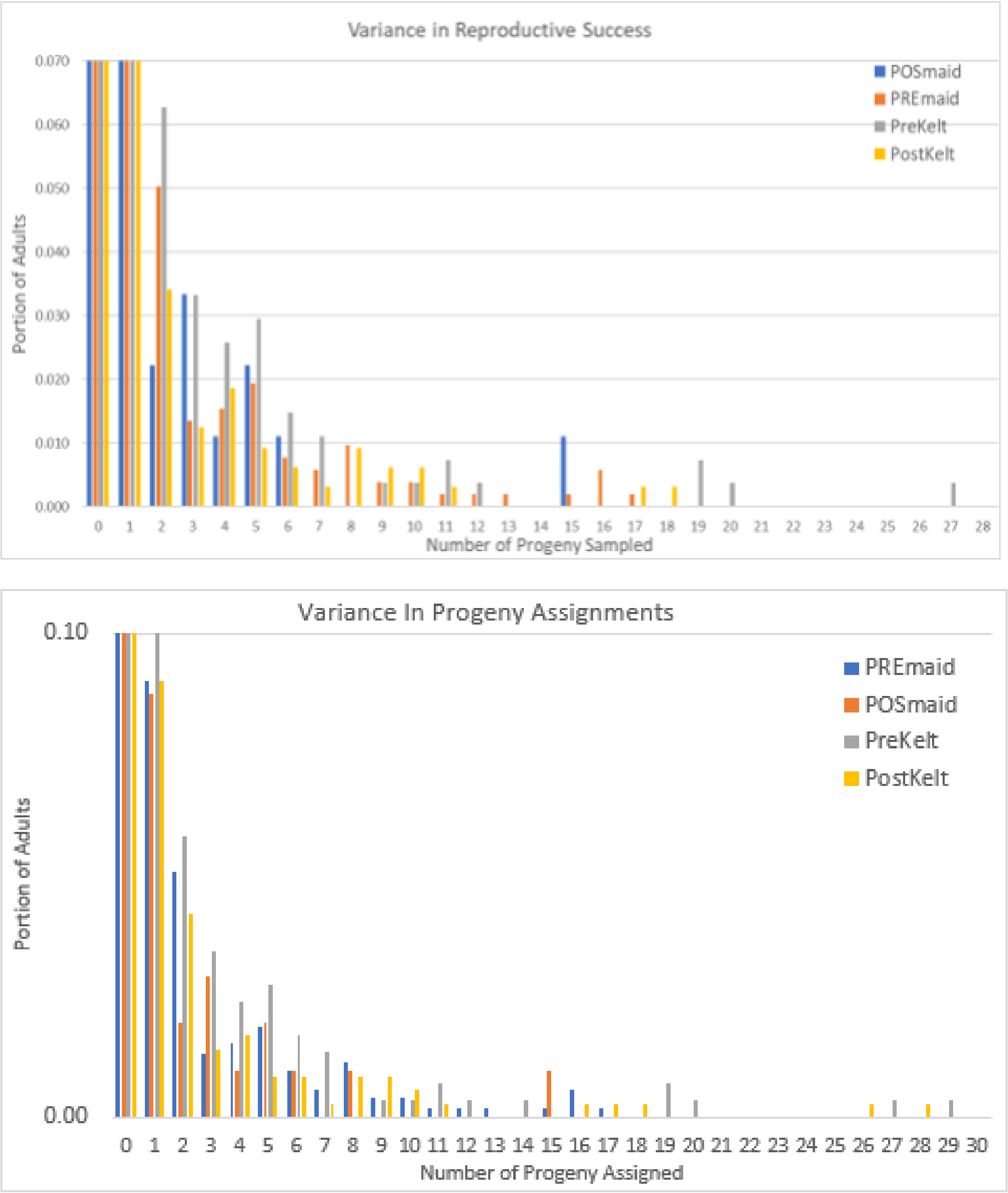


Figure 2. 4. Variation in reproductive success of maiden and kelt spawners. Both scales show the number of progeny across the portion of adults genotyped. The second part of the figure has a truncated y-axis to better demonstrate the variability based on number of progeny. POSmaid= Post spawn maiden event, PREmaid= Maiden event collected as pre-spawner, PreKelt= first spawning event for reconditioned kelt, and PostKelt=second spawning event for reconditioned kelt see Monitoring Methods, Genetic Analysis.

The number of genotyped parents confirmed to have entered either Satus or Toppenish creeks is shown in Table (2.6). Pre-spawn maidens have the greatest number of samples with a total of 284 males and 532 females. Post-spawn maidens have only 19 males and 103 females overall. Reconditioned kelts have 26 male and 293 female Event-1 detections and 30 male and 356 female Event-2 detections. The number of fish increase each year but are limited by the number of kelts that can be collected, and mortality during the reconditioning process. The low number of kelt males is likely due to female skewed returns (Frederiksen et al. 2019), and male spawners staying on spawning grounds seeking additional spawning opportunities until they likely perish from exhaustion or predation.

Table 2. 6. Number of genotyped adults with PIT detections. Only fish with both a PIT tag detection and genotypes were included in the parentage results.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**All**
Pre-Spawn Maidens	Male	38	46	57	79	6	21	6	18	13	284
Post-Spawn Maidens	Male	4	1	7	2	1	1	1	1	1	19
Reconditioned Kelt Event-1	Male	3	13	7	1	0	0	1	-	1	26
Reconditioned Kelt Event-2	Male	5	3	13	8	1	0	0	-	-	30
Pre-Spawn Maidens	Female	88	70	92	141	46	37	20	23	15	532
Post-Spawn Maidens	Female	12	13	38	9	2	5	5	6	13	103
Reconditioned Kelt Event-1	Female	15	43	51	22	13	44	47	35	23	293
Reconditioned Kelt Event-2	Female	73	19	38	56	26	15	44	49	36	356

The number of progeny assigned to each class of fish is shown in Table (2.7). The majority of assignments are to pre-spawn maidens with 261 juveniles assigned to males and 458 juveniles assigned to females. We had a great year finding kelt progeny in 2021 with 98 assigning to kelt parents. All other classes of fish have lower numbers assigned as a function of the lower representation in the number of adult fish both detected and genotyped.

Table 2. 7. Number of progeny assigned to each type of spawning event within individual years and across all years. Progeny assigned to adults without tributary PIT tag detections are not included here.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**All**
Pre-Spawn Maidens	Male	13	17	24	120	13	34	8	13	19	261
Post-Spawn Maidens	Male	1	0	0	4	7	1	0	0	0	13
Reconditioned Kelt Event-1	Male	5	6	4	1	0	0	3	NA	0	19
Reconditioned Kelt Event-2	Male	3	4	1	0	7	NA	NA	NA	NA	15
Pre-Spawn Maidens	Female	43	41	26	165	76	42	35	13	17	458
Post-Spawn Maidens	Female	8	1	27	2	0	9	9	1	8	65
Reconditioned Kelt Event-1	Female	5	17	32	17	63	67	112	30	60	403
Reconditioned Kelt Event-2	Female	18	12	16	40	39	38	58	9	98	328

The average number of progeny per parent is shown in Table (2.8). Numbers varied greatly between both years and between classes in within years. Males had ranges between 0.00 which happened in numerous years and 7.00 progeny per parent which occurred in 2017. While females had ranges from low 0 for post spawn maidens to almost 5 for kelts first time spawning event both occurring in 2017.

Table 2. 8. Average number of progeny per parent sampled for each category of fish. Results are reported as the average number of juvenile detections within each spawning event.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**All**
Pre-Spawn Maidens	Male	0.34	0.37	0.42	1.52	2.17	1.62	1.33	0.72	1.46	1.11
Post-Spawn Maidens	Male	0.25	0.00	0.00	2.00	7.00	1.00	0.00	0.00	0.00	1.14
Reconditioned Kelt Event-1	Male	1.70	0.46	0.57	1.00	NA	NA	3.00	NA	0.00	1.12
Reconditioned Kelt Event-2	Male	0.60	1.33	0.08	0.00	7.00	NA	NA	0.00	NA	1.50
Pre-Spawn Maidens	Female	0.50	0.59	0.28	1.17	1.65	1.14	1.75	0.57	1.13	0.97
Post-Spawn Maidens	Female	0.70	0.08	0.71	0.22	0.00	1.80	1.80	0.17	0.62	0.67
Reconditioned Kelt Event-1	Female	0.30	0.40	0.63	0.77	4.85	1.52	2.38	0.86	2.61	1.59
Reconditioned Kelt Event-2	Female	0.20	0.63	0.42	0.71	1.50	2.53	1.32	0.18	2.72	1.14

Relative reproductive success for each category of fish is seen in Table (2.9). Variance in the number's parallels that of the average number of progeny per parent with higher variance seen in males.

Table 2. 9. Relative reproductive success for each category of fish. Results are normalized to the Pre-Spawn Maiden even within in year.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**AVG**
Pre-Spawn Maidens	Male	1	1	1	1	1	1	1	1	1	1
Pos-Spawn Maidens	Male	0.73	0	0	1.32	3.23	0.62	0	0	0	0.66
Reconditioned Kelt Event-1	Male	4.87	1.25	1.36	0.66	NA	NA	2.25	NA	0	1.73
Reconditioned Kelt Event-2	Male	1.75	3.61	0.18	0.00	3.23	NA	NA	NA	NA	1.46
Pre-Spawn Maidens	Female	1	1	1	1	1	1	1	1	1	1
Pos-Spawn Maidens	Female	1.36	0.13	2.51	0.19	0	1.59	1.03	0.29	0.54	0.85
Reconditioned Kelt Event-1	Female	0.68	0.67	2.22	0.66	2.93	1.34	1.36	1.52	2.30	1.59
Reconditioned Kelt Event-2	Female	0.50	1.08	1.49	0.61	0.91	2.23	0.75	0.32	2.40	1.14

Lifetime reproductive success (LRS) of reconditioned kelts is shown in Table (2.10). Male kelt LRS varied between 0.0 and 4.1 times that of fish sampled as pre-spawn maidens within the same year. Across years 2013-2021 male kelts have an LRS of 1.72. Female kelt LRS had annual variation between 1.2 and 4.7 with an average of 2.67.

Table 2. 10. Lifetime reproductive success estimate for male and female Reconditioned kelts. Additive effects of Reconditioned Kelt Events 1 and 2 as normalized to the Pre-Spawn Maiden event.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**AVG**
Reconditioned Kelt Lifetime	Male	3.4	4.1	0.8	1	3.2	0	3	0	0	1.72
Reconditioned Kelt Lifetime	Female	1.2	1.80	3.7	1.3	3.8	3.6	2.1	1.80	4.7	2.67

The presence of kelt offspring demonstrates that reconditioned kelts successfully spawn in the wild. Lifetime reproductive success of female reconditioned kelts was calculated to be 2.41 times that of the pre-spawn maidens. This is similar to findings by Seamons and Quinn (2010) who theorized and found that lifetime reproductive success of natural repeat spawners should scale with the number of breeding spawners. We specifically found that the relative reproductive success of the second spawning event (post reconditioning) for female reconditioned kelts (1.14) is slightly better than that of putative first-time spawners and demonstrates the potential to boost numbers additively over their first spawning event.

The 2021 spawning event was the ninth consecutive year that we successfully assigned multiple progeny to reconditioned kelts. The methodology of focusing sampling efforts on age-0 fish in areas that anadromous spawning was expected to have occurred, and an increased sampling rate of juvenile has resulted in an increase in the number of successful assignments to both maiden and kelt fish. Future sampling will continue to focus on age-0 fish in areas that spawning was expected to have occurred. We will also start to incorporate analysis of adult offspring using upstream migrants sampled at Prosser Dam or the Chandler facility.

Reconditioned kelt steelhead have demonstrated that they are capable of spawning in the wild. With additional sampling in future years, including adult to adult estimates, we hope to have more accurate numbers and modeling potential. Current data shows that reconditioned kelt steelhead contribute to the productivity of the natural population on a scale similar to that of natural kelts, which will help to preserve this important life history in the Columbia River Basin. We anticipate that we will submit a manuscript on reproductive success in an accredited scientific journal by 2023.

What have we learned about kelt physiology and how does that inform decisions?

Previously, little was known about post-spawning physiology in iteroparous salmonids. There is no evidence for anything like the programmed post-spawning mortality that occurs in the semelparous members of *Oncorhynchus.* Iteroparity is the ancestral trait of *Oncorhynchus* (Crespi and Teo 2002), and the capacity for iteroparity is viewed as a trait of the species *O. mykiss* (Seamons and Quinn 2010). However, individuals may or may not be able to successfully express this trait. Post-spawning kelts experience high mortality due to injuries, susceptibility to pathogens, and bioenergetic exhaustion, as well as anthropogenic changes in the river corridor (Colotelo et al. 2014; Keefer et al. 2018; Keefer et al. 2008). For kelts that survive, little is known about the physiology of gonadal recrudescence, and questions regarding the reproductive status of reconditioned kelts were identified as critical uncertainties during earlier ISRP reviews of the project (ISRP 2011). To fill these knowledge gaps, beginning in 2009, we initiated a research program to investigate the physiology of post-spawning steelhead, with the goal of providing a scientific basis for maximizing the success of steelhead kelt reconditioning programs. Research covered the broad areas of energetics and survival; feeding, digestion and growth; stress; smoltification; disease; and reproduction. We conducted studies using wild kelts, hatchery-origin kelts from Dworshak National Fish Hatchery (DNFH), and post-spawning rainbow trout. Depending on the goals and the experimental system, we used both observational and manipulative experimental designs. Our main methods were non-lethal blood sampling and measurement of plasma hormones and other factors related to physiological status.

Energetics and Survival

Studies conducted by the Department of Fish and Wildlife Sciences at the University of Idaho have described aspects of energy depletion during spawning migration, sexual maturation and kelt emigration in Snake River steelhead (Penney and Moffitt 2014b, 2015). As expected, energy was progressively depleted from early migration to the kelt stage. Muscle lipids were used more rapidly than muscle protein. Muscle lipid use appeared to slow during fall and winter. Liver lipid stores were depleted more rapidly than muscle stores, and saturated and monounsaturated fatty acids were depleted more rapidly than polyunsaturated fatty acids, which may reflect conservation of polyunsaturated fatty acids due to their role in cell membrane structure and function. At the kelt stage, fish that were rated in “better condition” based on visual appearance had greater energy reserves, consistent with findings on survival in captive reconditioning and natural repeat spawning rates (Hatch, et al. 2013b; Keefer et al. 2008). Kelt length was positively correlated with energy density and muscle protein, suggesting greater energy reserves in larger fish, consistent with a decrease in resting metabolic rate with increasing body size (Clarke and Johnston 1999).

Most mortality in kelt reconditioning occurs over the initial interval after fish are captured after spawning or spawned in captivity (Hatch et al. 2013b; Jenkins, et al. 2020). In the DNFH hatchery, based off kelts undergoing reconditioning, a regression model analyzing factors associated with survival over the first 70 days after spawning found that plasma osmolality and triglyceride levels were positively associated with survival (Fig. 2.5) (Jenkins et al. 2020). In contrast, static measures of energy reserves such as condition factor and muscle lipid level were not significantly associated with survival, and neither were the reproductive measures size-adjusted total egg mass (TEM) or plasma estradiol (E2) level. The osmolality result indicates that osmoregulatory failure in freshwater is a major cause of mortality in kelts, similar to findings on predictors of pre-spawn mortality in Fraser River sockeye salmon (Jeffries, et al. 2011). The plasma triglyceride result indicates that the functioning of physiological systems enabling access to lipid stores is essential for survival. Measures that reflect functioning of physiological systems performed better as indicators of fish condition than static measures such as condition factor. Kelt use of muscle protein stores were not assessed in this study, and would be a potential future direction, as use of protein stores for energy can be significant in salmonid physiology (Mommsen 2004).

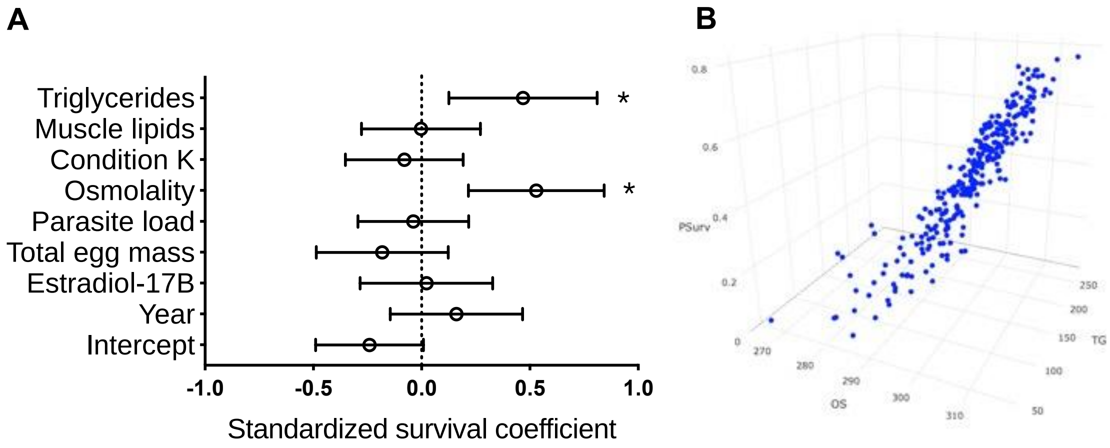


Figure 2. 5. (A) Standardized coefficients ([x-mean]/SD) with 95% confidence intervals of potential predictors of survival in a multiple logistic regression model of female steelhead trout at Dworshak National Fish Hatchery, Ahsahka, Idaho to 70 days after first spawning in 2015 and 2016 (n = 291). Potential predictors were measured at spawning. Positive coefficients indicate a greater probability of post-spawning survival. Coefficients with confidence intervals that do not overlap zero were statistically significant (P < 0.05) and are marked with an asterisk. (B) Post-spawn survival probabilities (PSurv) to 70 days, as predicted by plasma triglycerides (mg/dL) (TG) and plasma osmolality (mmol/kg) (OS) measured at first spawning in 2015 and 2016 (n = 291). From Jenkins et al. 2020.

Feeding, Digestion and Growth

Growth is minimal over the period after spawning in both hatchery and wild kelts (Jenkins, et al. 2019; Pierce, et al. 2017). Many fish continue to lose weight over the first 10 weeks after spawning, whereas even among fish with positive growth, growth rates are 5 to10-fold lower than later during reconditioning (Jenkins et al. 2019). Suppressed growth during this period is due to minimal feed consumption, although fish are offered highly palatable feeds such as krill, multiple times throughout the day. However, in kelts that were fasted for the first 10 weeks after spawning, growth rates immediately increased after food was made available, and exceeded growth rates of fish that had been fed throughout, a compensatory growth response to the fast (Fig. 2.6) (Hatch et al. 2020; Jenkins, et al. In preparation). This implies that there is a process of recovery from spawning that must occur before feeding and growth can begin in earnest, and that this process does not necessarily depend on feeding. Feeding motivation and feed consumption are strongly reduced during the recovery period. The physiology underlying the recovery period is not well understood. However, it is reasonable to hypothesize that it may relate to restoration of digestive system function after a prolonged fast, and reversal of the catabolic state associated with elevated levels of the stress hormone cortisol that occurs during the spawning period in salmonids (Barry, et al. 2010; Dickhoff 1989; Schreck, et al. 2001).

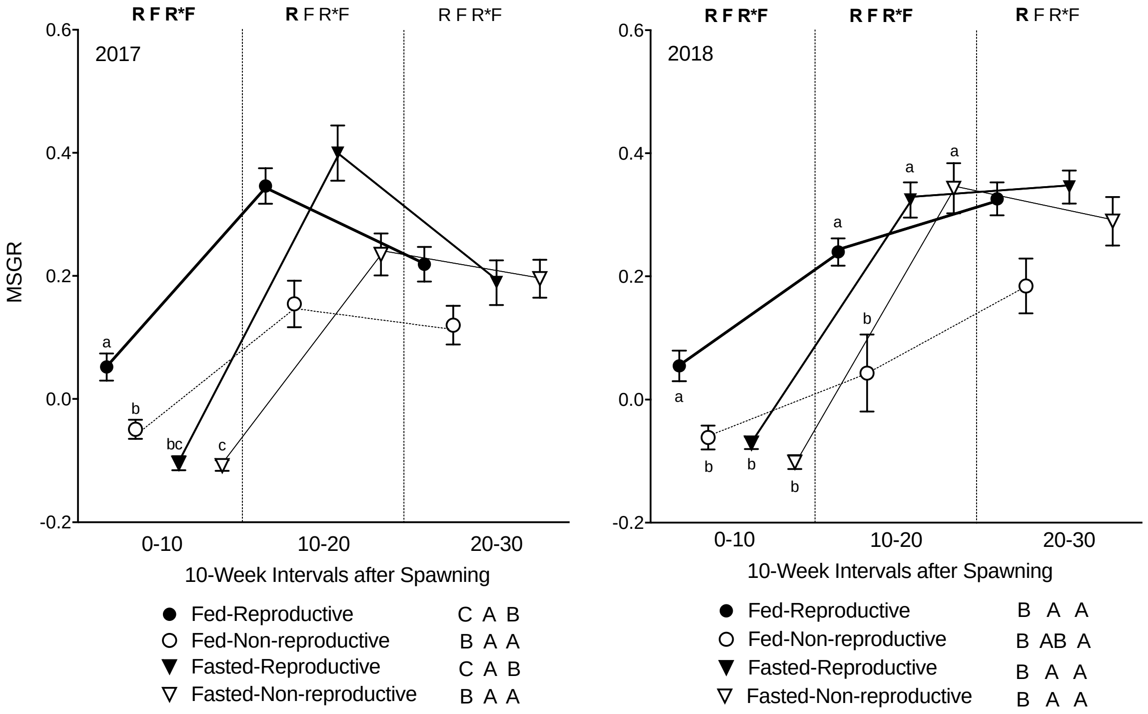


*Figure 2. 6. Specific growth rate in mass (MSGR) in female steelhead trout sampled at 10-week intervals starting at first spawning. Food was withheld from Fasted fish for the first 10 weeks after spawning. Symbols indicate mean and bars indicate SEM. Above each sampling point, bolded letters indicate significance (R = Reproductive status, F = Fasting Treatment, R*F = Interaction effect). Rows of letters below the figure indicate significant differences over time within each treatment group. Years are shown separately due to a significant year effect at week 20-30. With years combined, Fasted fish showed significantly higher MSGR than Fed fish over week 10-20. From Jenkins et al. In Preparation.*

Maintenance of a functioning digestive system is energetically costly, and animals that routinely fast for prolonged periods often reduce this cost by atrophy of the digestive epithelia (Secor, et al. 2002; Zaldua and Naya 2014). The gut-somatic index decreases linearly over time in fasted juvenile rainbow trout with a loss of ~40% of the relative mass of the gut over 147 days of fasting (Simpkins, et al. 2003). In Atlantic salmon that fasted for 50 days, restoration of the gut upon refeeding required at least one week, during which feed intake was reduced (Krogdahl and Bakke-McKellep 2005). As would be expected after their prolonged fasting spawning migration, the stomach and intestinal epithelia are atrophied in post-spawning summer run steelhead trout, and degenerative changes are found in the liver (Penney and Moffitt 2014a). Some kelts showed evidence of regeneration of the gut epithelia, although it was not clear whether this was a consequence of or preparation for feeding activity. In some species, food in the lumen of the gut stimulates restoration of function (Secor et al. 2002), and force feeding has been successfully employed in Atlantic salmon kelt reconditioning projects (Eales, et al. 1991) and with precocious Chinook salmon parr (Bernier, et al. 1993). To determine if this might be effective with steelhead kelts, we force fed DNFH hatchery origin kelts after spawning (Hatch, et al. 2012). However, this resulted in decreased survival, and the stomachs of mortalities contained partially digested food 35 days after force feeding. This suggests that there was a delay in digestion associated with the restoration of digestive function. The metabolic cost of restoration of gut function may have outweighed benefits obtained from the food. Based on these results, force feeding does not appear to be a promising method for steelhead kelt reconditioning.

The decreased feeding motivation of kelts during the period after spawning may be due to changes in the levels of orexigenic and anorexigenic endocrine and neuroendocrine factors. One such factor is ghrelin, a strongly orexigenic (appetite stimulating) hormone produced by the stomach (Kojima and Kangawa 2005). Unlike other appetite stimulating factors, ghrelin crosses the blood-brain barrier. In humans, ghrelin is reduced in stomach atrophy (Osawa, et al. 2005). Thus, it is possible that steelhead kelts may have reduced appetite due to decreased plasma ghrelin caused by stomach atrophy. Ghrelin is highly conserved, and commercially available mammalian ghrelins are effective in fish species. In most trials in fish, ghrelin administration increased food intake (Riley, et al. 2005; Shepherd, et al. 2007; Unniappan and Peter 2004, 2005). However, in several studies, appetite has not been stimulated (Jonsson, et al. 2007), or has even been suppressed (Jonsson, et al. 2010). To determine if ghrelin has potential as an appetite stimulant in kelt steelhead, we tested ghrelin administration in juvenile rainbow trout (Branstetter, et al. 2010). Fish were intraperitoneally implanted with Alzet micro-osmotic pumps that administered rat ghrelin or vehicle at a constant rate over time and tracked for 20 days. Feed consumption and growth were quantified. Fish in the ghrelin treated tanks exhibited unusual behavior. During feeding, fish were at the top of the tank, with their backs out of the water, and were highly active. Ghrelin treated fish pursued pellets, but often did not ingest them. Fish in the control treatment tanks did not show these behaviors. Feed intake in ghrelin treated tanks was lower than controls, and growth rates were lower than controls. Thus, ghrelin appeared to inhibit appetite and growth under our experimental conditions. As well as stimulating appetite, ghrelin stimulates corticotrophin-releasing factor (CRF) neural circuits in the brain (Jonsson et al. 2010). Stimulation of the CRF system produces increases in activity level and suppresses feed intake (Lowry and Moore 2006), consistent with our observations on the behavior of ghrelin treated fish. These findings suggest that the effect of ghrelin administration may depend on the relative magnitude of the effects mediated by these different mechanisms. Due to the complexities involved, we decided not to pursue ghrelin treatment any further.

Stress

The stress axis or hypothalamic-pituitary-interrenal (HPI) axis is the central endocrine pathway mediating the stress response in fish (Barton 2002; Wendelaar Bonga 1997). Activation of the HPI axis results in an increase in circulating corticosteroids, such as cortisol (Medeiros, et al. 2010; Wendelaar Bonga 1997). High circulating cortisol levels are found during the final stages of gonadal maturation and during spawning in salmonids (Laidley and Leatherland 1988). Cortisol initially plays an adaptive function during stress, but chronic elevations of cortisol contribute to the deleterious effect of chronic stress (Barton and Iwama 1991). Chronic cortisol elevation induces a catabolic state in which protein synthesis is inhibited and energy is mobilized from carbohydrate, protein and lipid stores and immune function is suppressed (Mommsen, et al. 1999; Wendelaar Bonga 1997) (Schreck 1981, 1990). Elevated cortisol levels and the resulting catabolism, anorexia, and suppression of immune function are proposed to cause post-spawning death in the semelparous Oncorhynchus species (Dickhoff 1989). In addition, activation of the stress axis inhibits the reproductive endocrine axis (Barton 2002; Donaldson 1990). Therefore, we hypothesize that survival, recovery from spawning, and gonadal recrudescence in steelhead kelts may depend on a post-spawning reduction in circulating cortisol levels. Although basal (unstressed) circulating cortisol levels are difficult to measure due to the rapid response to capture stress, we have indirect evidence that it takes approximately 6-10 weeks for cortisol levels decrease after spawning in rainbow trout. Plasma levels of the growth stimulatory hormone insulin-like growth factor-1 (IGF1), for which we have recently established a laboratory assay (Medeiros, et al. 2020), increased after spawning for 6-8 weeks in rainbow trout, and did not respond to rations until 8 weeks (Hatch et al. 2020). Consistent with its catabolic function, cortisol suppresses IGF1 (Pierce, et al. 2005a). Liver gene expression levels of IGF binding protein 1b (IGFBP1b) decreased for 8-10 weeks after spawning in rainbow trout (Fig. 2.7) (Caldwell, et al. 2013). IGFBP1b is an indicator of stress and is directly induced by corticosteroids (Kajimura and Duan 2007; Pierce, et al. 2006). A fuller characterization of post-spawning changes in the HPI axis would be very useful.

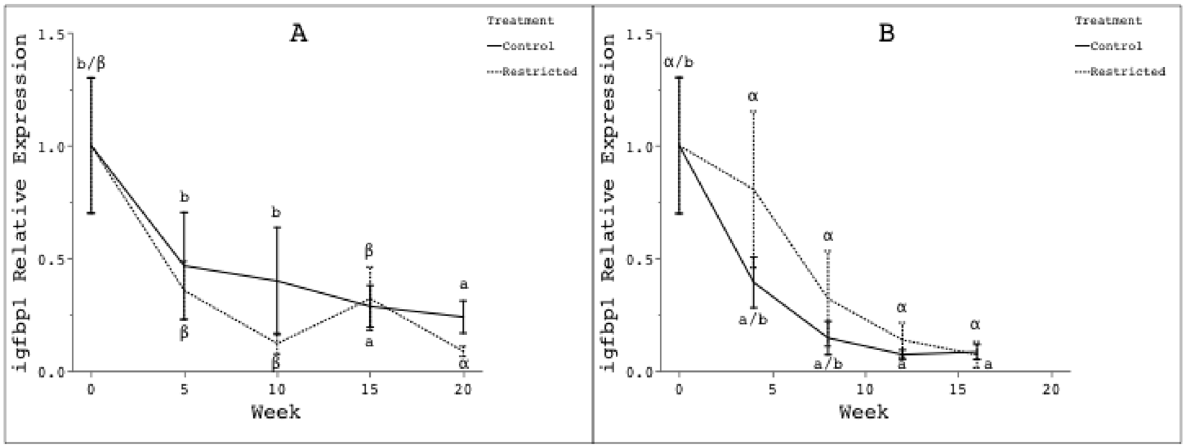


Figure 2. 7. Mean (±SEM) liver insulin-like growth factor binding protein 1 (igfbp1) mRNA levels over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. From Caldwell et al. 2013.

Along with studies describing the recovery from spawning, we conducted one manipulative study. In teleosts, most of the long-term changes in response to cortisol are mediated by cytosolic glucocorticoid receptors (GRs) located in target tissues, although other receptors exist. The GR antagonist mifepristone (RU486) blocks the physiological response to cortisol in salmonids and other teleosts (McDonald and Wood 2004; McDonald, et al. 2004; Medeiros, et al. 2014; Medeiros and McDonald 2013; Rodela, et al. 2009), interrupting the cortisol signaling cascade that leads to depressed appetite and the other undesirable effects of chronic stress. To determine if long term RU486 administration might speed up recovery from spawning, we tested if this treatment was effective in increasing growth in post-spawning female rainbow (Hatch, et al. 2017). Post-spawning rainbow trout were implanted with a time release formulation of RU486 in a mixture of vegetable shortening and vegetable oil, or vehicle alone. However, high mortality (50%) occurred over the 42-day course of the experiment, possibly associated with the poor condition of the fish available for the experiment and repeated handling. Mortality did not differ between treatment groups, and no differences were detected in post-spawning growth. This experiment needs to be repeated with a higher number of surviving fish before any conclusions regarding the efficacy of the treatment can be made.

Smoltification

The ability to osmoregulate in saltwater increases with body size in salmonids, and consequently kelts would not be expected to need to undergo smoltification as a preadaptation to adjust to saltwater (Clarke and Hirano 1995). However, numerous other physiological changes occur during smoltification, which may be necessary for fish to successfully make the transition to the ocean (McCormick 2012). Gill sodium-potassium ATPase activity increased in kelts as compared with spawning steelhead, however, plasma thyroxine levels were lower in kelts (Buelow and Moffitt 2015). Thus, it is unclear whether kelts undergo a preadaptation process similar to smoltification, since both factors typically increase during smoltification (McCormick 2012). Further study is needed to characterize smoltification related changes in steelhead kelts. Archival tagging studies have generally not found extensive mortality associated with ocean entry in steelhead kelts, although numbers are low (Hayes, et al. 2012; Miller, et al. 2021; Nielsen, et al. 2011; Null, et al. 2013). Plasma ion concentrations were higher in better condition steelhead kelts in the Clearwater River, suggesting that loss of the ability to osmoregulate in freshwater may be an early indicator of impending mortality (Buelow and Moffitt 2015). Our results in hatchery kelts reconditioned in freshwater confirm this supposition (Fig. 2.5) (Jenkins et al. 2020). Thus, based on the limited information currently available, it appears that freshwater osmoregulation is more critical to the survival of kelts than saltwater osmoregulation.

Disease

Immunosuppression and susceptibility to opportunistic pathogens are observed during spawning in multiple fish species. In steelhead kelts, these problems are exacerbated due to bioenergetic exhaustion and injuries sustained during spawning, downstream migration, and collection at dams and weirs. The crowded conditions the fish experience in culture also greatly facilitate disease transmission. The principal opportunistic pathogens that impact kelts are external *Saprolegnia* infestation, bacterial infections, and gill copepods. Consistent daily formalin treatment to control *Saprolegnia* outbreaks has been found to be essential to minimize mortality in kelt reconditioning. The installation of automated formalin treatment systems has resulted in reduced mortality at both the Prosser and Dworshak reconditioning sites. Prophylactic treatment of kelts at intake by injection with oxytetracycline, a broad-spectrum antibiotic, has been found to decrease mortality due to bacterial infection. In 2009, we did not treat fish at intake with oxytetracycline, due to concerns from a fish disease specialist employed by the US Fish and Wildlife Service about excessive antibiotic use, and experienced significantly higher mortality (Branstetter, et al. 2009).

Infestation of fish gills with freshwater copepod ectoparasites (Salmincola spp.) is a chronic problem in steelhead and Atlantic salmon kelt reconditioning (Hatch, et al. 2013a; McGladdery and Johnston 1988), which can cause mortality and suppress rematuration (Branstetter, et al. 2007; Duston and Cusack 2002). Treatment of kelts for parasitic copepods is necessary for successful reconditioning. The standard treatment for copepods in the Columbia River Basin had been ivermectin gavage (Johnson and Heindl 2001; Roberts, et al. 2004). However, neurotoxic effects of ivermectin have also been reported (Johnson, et al. 1993; O'Halloran, et al. 1992; Palmer, et al. 1987), and were also observed after ivermectin administration to kelts. We evaluated and implemented an alternative treatment using emamectin benzoate, which was more effective at controlling copepods and resulted in substantially reduced mortality (Fig. 2.8) (Branstetter, et al. 2011; Hatch, et al. 2014; Hatch et al. 2012).

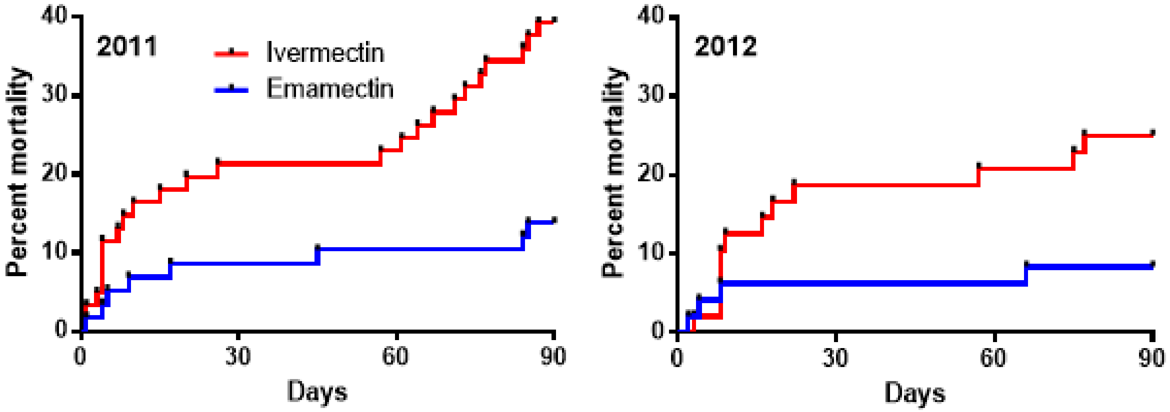


Figure 2. 8. Mortality of Prosser kelts after ivermectin or emamectin treatment at intake. Log-rank test mortality curve comparison, 2011: p = 0.0019; 2012: p = 0.0340. From Hatch et al. 2013b.

Reproduction

The bulk of the progress to date in research on kelt steelhead physiology has been in the area of female reproductive development. We have documented that the consecutive and skip spawning life histories are found in reconditioned kelts, established methods for screening fish for maturation status before release, and estimated the expected productivity benefit from released consecutive and skip spawners spawning in the wild. We have evaluated a hypothesized trade-off between reproductive investment in maiden spawning and post-spawning survival and post-spawning reproductive investment. As part of this work, we found interesting relationships between growth and nutritional status early during oogenesis and subsequent reproductive parameters at repeat spawning, shedding light on the physiological mechanisms regulating egg size and fecundity. Finally, we have investigated the timing of the rematuration decision and factors associated with it, establishing that initiation of rematuration occurs early during reconditioning, that it is associated with faster growth and better nutritional status, and that both physiological status at spawning and post-spawning feeding conditions influence the rematuration decision. This implies that, although optimal conditions in reconditioning can maximize consecutive spawning rates, pre-capture environmental conditions likely set a limit to consecutive spawning rates. We have found no evidence for functionally significant regulation of reproductive trajectory in steelhead at a second “permissive” decision window proposed for Atlantic salmon. Much of the progress in this area has been made possible by access to hatchery-origin kelts at DNFH.

The consecutive and skip spawning life histories are found throughout iteroparous seasonally spawning teleost species, and substantially impact fisheries management and conservation (Rideout, et al. 2005; Rideout and Tomkiewicz 2011). Consecutive spawners initiate gonadal rematuration on schedule to spawn at a one-year interval after initial (aka “maiden”) spawning, whereas skip spawners rest for a year, typically spawning at a two-year interval, although longer resting intervals are possible. In salmonids, post-spawning life histories are plastic: multiple repeat spawners can spawn at different intervals (Chaput and Jones 2006; Halttunen 2011; Hubley and Gibson 2011; Moore, et al. 2014; Riva-Rossi, et al. 2007). Larger, older fish, with a longer migration distance and/or later spawn timing are more likely to skip spawn (Chudyk 1976; Jonsson, et al. 1991; Keefer et al. 2018; Keefer et al. 2008; Moore et al. 2014). In general, both the presence of repeat spawners, and variation in repeat spawning interval increase population stability via the portfolio effect (Moore et al. 2014; Schindler, et al. 2010). Repeat spawners are also more fecund than maidens due to their larger size, multiplying their productivity benefit (Chaput and Jones 2006; Halttunen 2011). Early studies on wild steelhead kelts undergoing reconditioning confirmed that the consecutive and skip spawning life histories are found in reconditioned kelts (Fig. 2.9) (Pierce et al. 2017). Plasma levels of estradiol (E2), the principal female steroid, and vitellogenin (VG), a phospholipoprotein produced by the liver that functions to shuttle energy into the ovary during oogenesis, diverged into two groups by early fall: consecutive spawners had high levels of both factors, and potential skip spawners had lower levels. The migration patterns of released fish from the two categories concorded with their reproductive status, with consecutive spawners migrating upriver at higher rates, and skip spawners occasionally detected migrating upriver the following season. The time course of the increase in plasma E2 and VG was such that complete separation occurred by late July to mid-August. This enabled blood sampling and identification of consecutive and skip spawning individuals before release time, and appropriate management of the two life history types, which has now become a routine part of the project. Visual assessment of maturation status of Prosser kelts at release time was approximately 70% correct, which is not surprising given that this is five or more months before spawning.

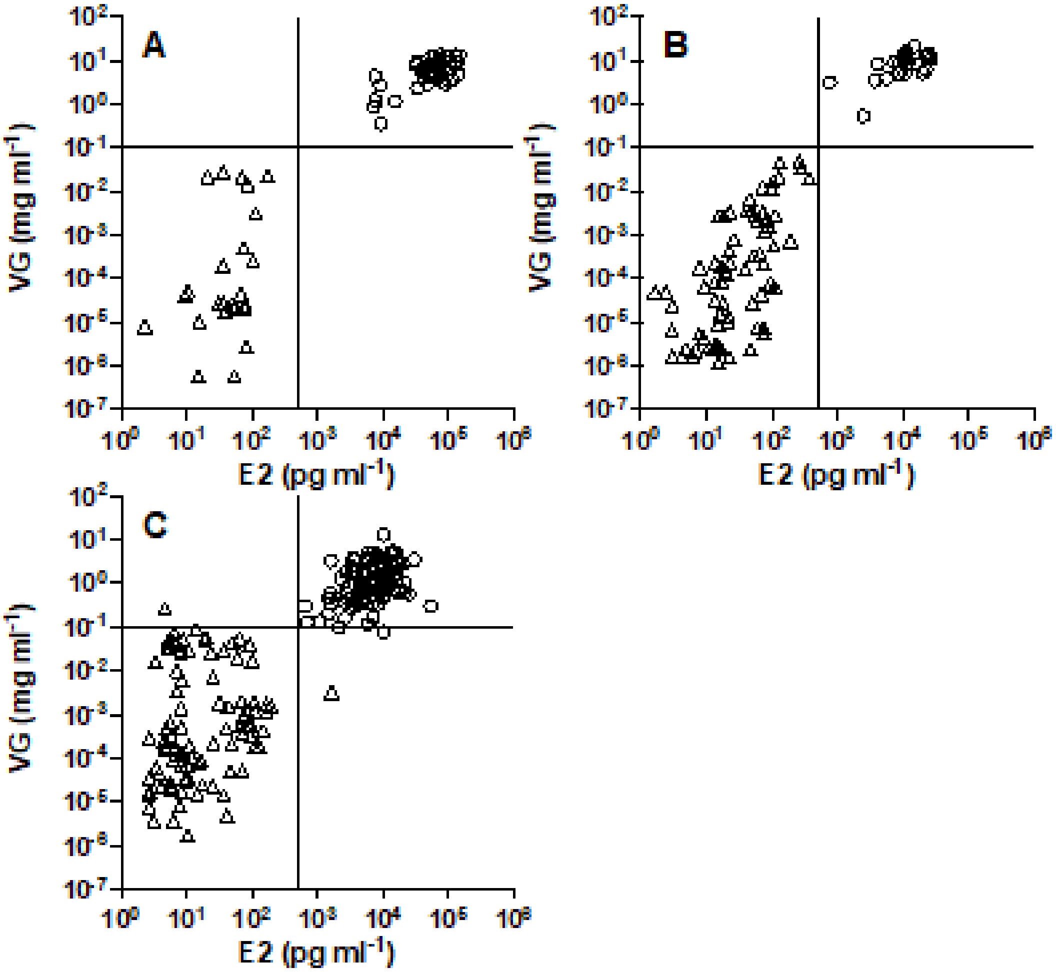


Figure 2. 9. Relationship of plasma levels of estradiol (E2) and vitellogenin (VG) in reconditioned female kelts at pre-release sampling in the fall of 2009 (A: 10/29/2009), 2010 (B: 10/13/2010), and 2011 (C: 10/13/2011). Fish were grouped by cluster analysis (Ward linkage, 2 groups specified, data standardized), and the resulting groups are indicated by circles (rematuring fish with high E2 and VG) and triangles (non-rematuring fish with low E2 and VG). Proposed threshold values for screening fish for maturation status by plasma E2 (500 pg ml^{-1}) and VG (0.1 mg ml^{-1}) level are indicated by lines. From Pierce et al. 2017.

The most significant potential criticism of kelt reconditioning projects, in our view, is a hypothesized reproductive life-history tradeoff between reproductive investment in maiden spawning and energy retained for subsequent survival and investment in repeat spawning. We believe that this is the issue underlying ISRP statements from more than decade ago suggesting that kelt reconditioning might select for reduced fitness (ISRP 2008). A recent adult-to-adult reproductive success study on Hood River steelhead found that iteroparous individuals had reduced success on their maiden spawning, which was interpreted as being due to reduced maiden spawning investment (Christie, et al. 2018). However, there are alternative explanations for these results, and the existence and magnitude of any potential trade-off have not been directly assessed. Using the DNFH hatchery kelt model, we were able to directly test these predictions, both in terms of survival and investment in repeat spawning. Size-adjusted maiden spawning total egg mass (TEM), a reasonable measure of reproductive investment, was not significantly related to survival after spawning (Fig. 2.5) (Jenkins et al. 2020). However, maiden TEM was the only factor that predicted consecutive spawning, and the relationship was positive: fish with greater maiden TEM were more likely to become consecutive spawners (Fig. 2.10) (Jenkins et al. 2020). Consecutive spawning requires greater investment of energy during the period after spawning than skip spawning, so this result shows that individual females with greater maiden reproductive investment also invest more in repeat spawning. As described in Stearns' analysis of life-history tradeoffs, this result implies that variation in condition between individuals is of greater importance than a tradeoff between current and future reproduction in determining reproductive investment, as found in other studies (Hendry, et al. 2000; Stearns 1992). Thus, if anything, kelt reconditioning is selecting for individuals that are best adapted to current conditions.

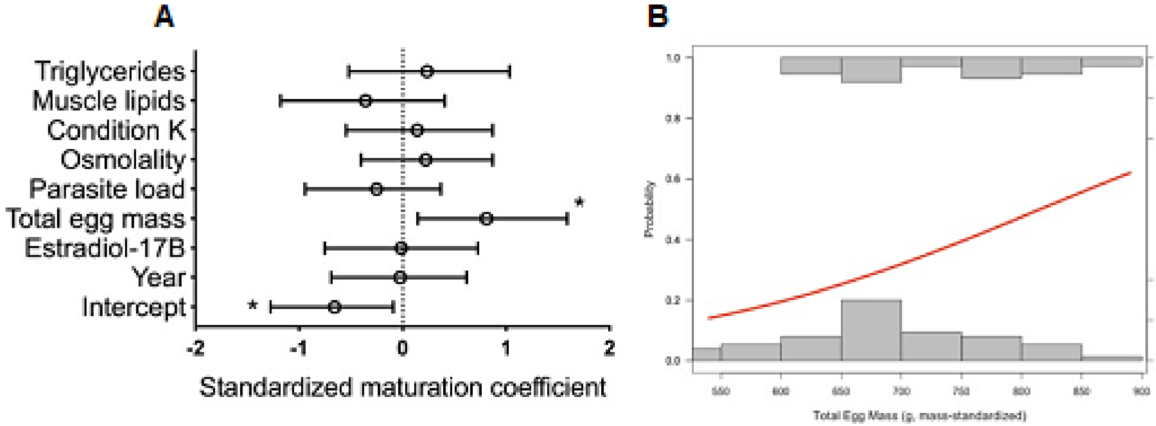


Figure 2. 10. (A) Standardized reproductive status coefficients ([x-mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of reproductive status in consecutive years of female steelhead trout (n = 60) following manual spawning in 2015 and 2016. Positive coefficients indicate greater probability of consecutive reproductive status. Coefficients with confidence intervals that do not overlap zero were statistically significant (P < 0.05) and are marked with an asterisk. (B) The probability of reproductive status in consecutive years in reconditioned female steelhead trout at Dworshak National Fish Hatchery, Ahsahka, Idaho, in relation to total egg mass at first spawning (g, mass-standardized, n = 60). The bars at the top and bottom of the graph represent the number of fish that became reproductively active or remained non-reproductive following first spawning, respectively, for each range of total egg mass. From Jenkins et al. 2020.

As well as evaluating potential relationships of maiden spawning reproductive performance to post-spawning outcomes, the DNFH hatchery kelt model enabled us to directly assess repeat spawning reproductive performance in reconditioned kelts. Based on issues with spawn timing in captive broodstock programs, we wanted to determine whether ovulation timing was shifted in reconditioned kelts. Consecutive spawning fish ovulated the same week on average as their maiden spawning, whereas skip spawners ovulated 3 weeks earlier (Fig. 2.11) (Jenkins, et al. 2018). Earlier migration timing for natural skip spawners versus maiden or consecutive spawners has been reported in Atlantic salmon and steelhead (Keefer et al. 2018; Niemela, et al. 2006). Ovulation timing for both consecutive and skip spawners was within the range for maiden spawners in this population. Consecutive spawners had 1.28-fold, and skip spawners 1.52-fold, the expected productivity of maiden spawners, based on total egg mass. When standardized for fish size, consecutive spawners had relatively smaller and skips spawners relatively larger eggs, than maiden spawners. These results are similar to findings in repeat-spawning Atlantic salmon (Reid and Chaput 2012). Interestingly, measures of energy balance such as growth rates and plasma triglyceride level correlated positively with size-standardized egg size at 10 weeks after spawning and with size-standardized total egg mass and fecundity at 20 weeks after spawning (Jenkins et al. 2020). This suggests that egg size and fecundity are set based on physiological condition during early- to mid- oogenesis, and that egg size is set first, in repeat spawning steelhead and potentially in other salmonids. If generally true, this might inform evolutionary models on optimal egg size and the egg size/fecundity tradeoff (Einum, et al. 2002; Hendry and Day 2003; Kinnison, et al. 2001), and provide a mechanism linking environmental conditions with reproductive performance.

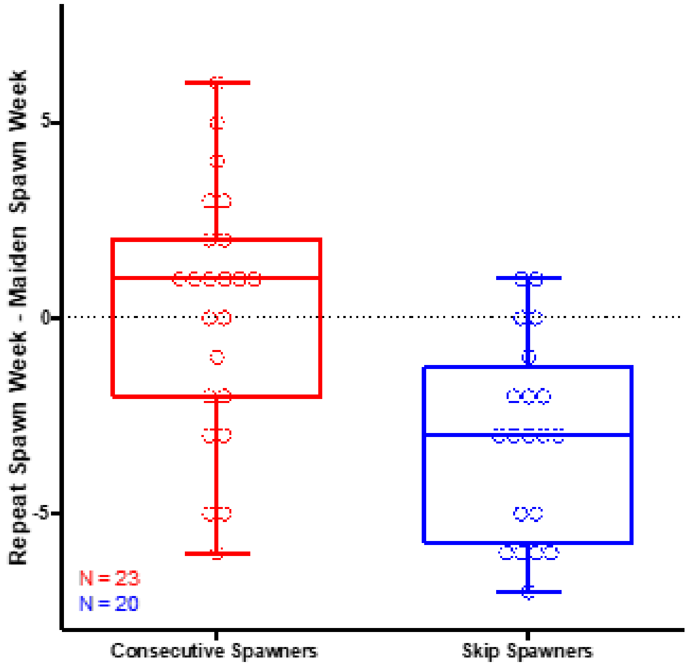


Figure 2. 11. Spawn week fidelity in repeat spawing female steelhead trout from the Clearwater River, ID sampled between 2013-2015. Boxes indicate the interquartile range, the line indicates the median, whiskers show the data range, and all points are shown. Repeat spawn week did not differ significantly from maiden spawn week in consecutive spawners (one sample t-test, t_{22} *= 0.2630, p = 0.7950), but was significantly earlier in skip spawners (one-sample t-test,* t_{19} *= 5.4978, p<0.0001), and repeat spawn week was significantly earlier in skip spawners than consecutive spawners (two-sample t test,* t_{41}*=3.671, p=0.0007). From Jenkins et al. 2018.*

Because of the importance of consecutive and skip spawning in steelhead kelt reconditioning programs, we have sought to better understand the physiological decision underlying the development of these two life histories. Work in this area has been guided by the critical period model of salmonid maturation decisions. This was developed for Atlantic salmon (*Salmo salar*) and proposes that the initial maturation decision takes place during a seasonally defined critical period approximately one year before spawning and is gated by stores of metabolic fuels (Thorpe 2007; Thorpe, et al. 1998). The aspect of metabolic fuel storage that regulates entry into a reproductive cycle is not known but is functionally defined as either the absolute level or the rate of change of body size (growth) and/or lipid reserves (Taranger, et al. 2010; Thorpe 2007). Endocrine signaling factors associated with lipid metabolism and/or growth are presumed to provide the mechanistic link between energetic status and the reproductive endocrine axis (Taranger et al. 2010). A second “permissive” critical window is proposed to occur approximately 6 months later, during which maturation can be arrested if energy stores are not sufficient. Thus, consecutive spawners must initiate rematuration during the first window and continue at the second, whereas skip spawning results from either not initiating at the first window or arresting at the second. However, the timing of the critical periods and the relevant aspect of metabolic fuel storage and network of associated signaling factors are not known in detail in any species. In steelhead kelts, the extreme energy depletion from maiden spawning migration and the maiden spawning event itself would be expected to influence this decision process.

Evidence from wild kelts, our DNFH hatchery kelt model, and the post-spawning rainbow trout model shows that the functionally important critical period occurs early during the post-spawning period and may extend to before spawning. Plasma levels of estradiol (E2), the principal female reproductive steroid in salmonids, reliably diverged between consecutive and skip spawners at 20 weeks post-spawning in DNFH kelts (Fig. 2.12) (Jenkins et al. 2019). The timing of the increase was somewhat sooner at approximately 13 weeks in wild kelts (Pierce et al. 2017), whereas the difference occurred at 8-10 weeks in post-spawning rainbow trout (Caldwell, et al. 2014). The difference in timing likely relates to metabolic rate, which would be slower in the DNFH fish due to their larger size and the colder water temperatures at this site due to earlier spawn timing. Elevations in plasma E2 only occur after upstream events in the brain-pituitary-ovary-liver endocrine axis (Lubzens, et al. 2010; Taranger et al. 2010; Wootton and Smith 2015). The amount of time required for these events is not known with any certainty; however, in maturing coho and Atlantic salmon maiden females, increases in plasma E2 were first detected 10 months before ovulation (Andersson, et al. 2013; Campbell, et al. 2006). Thus, the increase in plasma E2 sets a later bound to the initiation critical period. Examination of individual profiles of E2 over time do not show more than a few individuals where E2 initially increases, and then decreases after the time of the purported second permissive critical period (Jenkins et al. 2019; Pierce et al. 2017). Thus, we do not find support for functionally significant regulation of rematuration during this window in steelhead kelts. This part of Thorpe’s model makes sense for fall spawners like Atlantic salmon, which may need to reassess energy reserves after the winter. However, summer steelhead would need to begin migration from ocean feeding areas toward river mouths in mid-summer, and thus the second critical period makes little sense in the context of the summer steelhead life history.

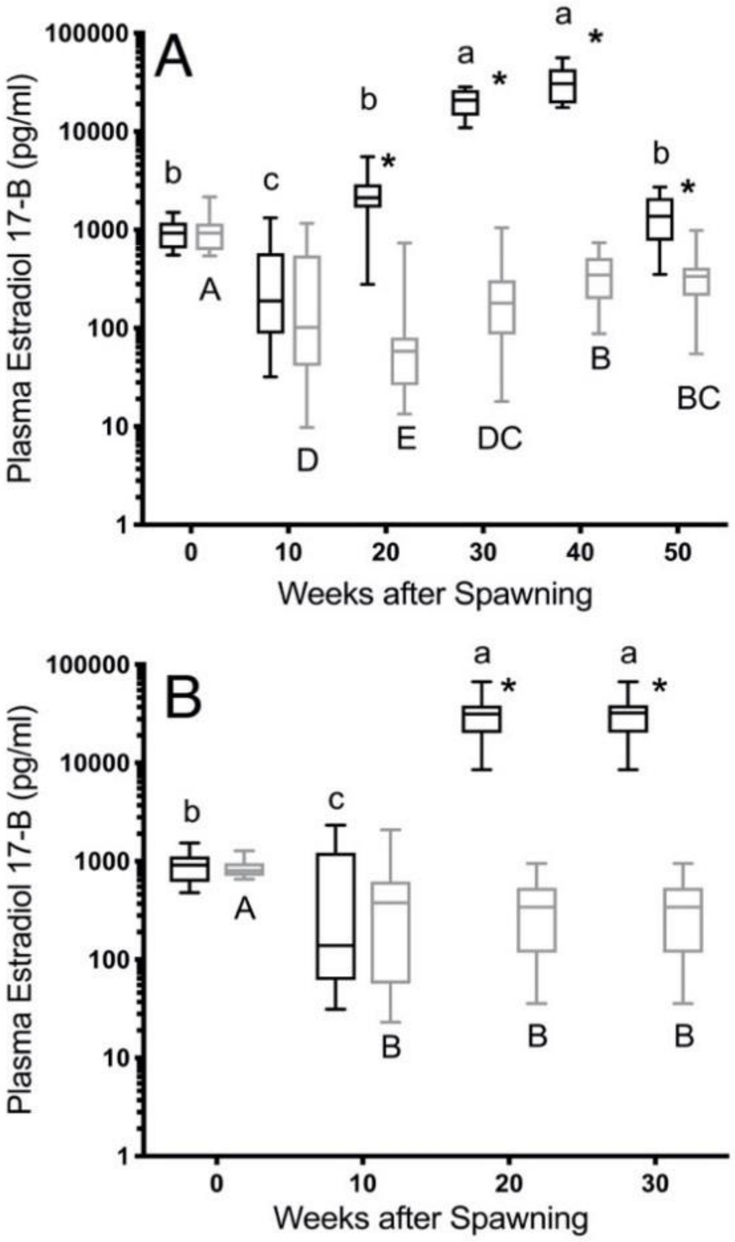


Figure 2. 12. Plasma estradiol-17β concentrations in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following maiden spawning. Females were grouped as rematuring (red: 2015, N=13; 2016, N=12) or non-rematuring (blue: 2015, N=29; 2016, N=18). Box heights indicate interquartile range, horizontal lines within indicate the median, and whiskers show the data range. Time points within a rematuration group sharing the same letter do not differ significantly (One-Way ANOVA followed by Tukey's Multiple Comparison Test, $P<0.05$). Asterisks indicate significant differences between rematuration groups at each time point (T-test, $P<0.05$). From Jenkins et al. 2019.

Initiation of maturation as a consecutive spawner during the post-spawning period is associated with greater growth during this period, although growth is minimal during recovery from spawning in kelts and rainbow trout (Fig. 2.6) (Caldwell et al. 2013; Jenkins et al. 2019; Pierce et al. 2017). Plasma triglyceride levels are elevated in consecutive spawners at 10 weeks post-spawning, consistent with greater feeding during this period versus skip spawners (Jenkins et al.

2019). To clarify the relative roles of physiological condition at spawning and post-spawning feeding conditions on the consecutive maturation decision, we conducted an experiment in which DNFH hatchery kelts were fasted during the first 10 weeks after spawning. To explore the role of the growth hormone/insulin-like growth factor (GH/IGF) endocrine axis in reproductive decisions in steelhead kelts, we used recently developed assays in our laboratory (Medeiros et al. 2020) to measure plasma GH and IGF1 levels in this experiment. The GH/IGF axis is the principal physiological system that regulates growth in salmonids, as in other vertebrates (Norbeck, et al. 2007; Perez-Sanchez, et al. 2018; Wood, et al. 2005). During fasting and under other catabolic conditions, GH increases, and functions to mobilize stored energy (Bergan-Roller and Sheridan 2018; Bjornsson, et al. 2018; Norbeck et al. 2007; Pierce, et al. 2005b). Thus, elevated plasma GH can be used as an indicator of catabolic status. Some DNFH hatchery females in both the fed and fasted groups initiated rematuration as consecutive spawners. However, fish in the fasted group that were able to do this had significantly lower plasma GH levels at spawning than other groups, indicating that they were less catabolic at this time point (Fig. 2.13) (Jenkins et al. 2020). Plasma GH also increased during the fast as in subadult salmonids (Bjornsson et al. 2018; Pierce et al. 2005b). From this, we can conclude that 1) both metabolic state at spawning and post-spawning feeding conditions regulate consecutive spawning, 2) post-spawning feeding conditions were more important under our experimental conditions, 3) GH is a good indicator of metabolic state, and 4) the GH/IGF axis responds to nutritional status even after the prolonged fasting spawning migration of summer steelhead. These results imply that both pre-capture conditions and conditions in culture will affect consecutive spawning rate and suggest that steelhead show physiological adaptation to a period of continued fasting after spawning.

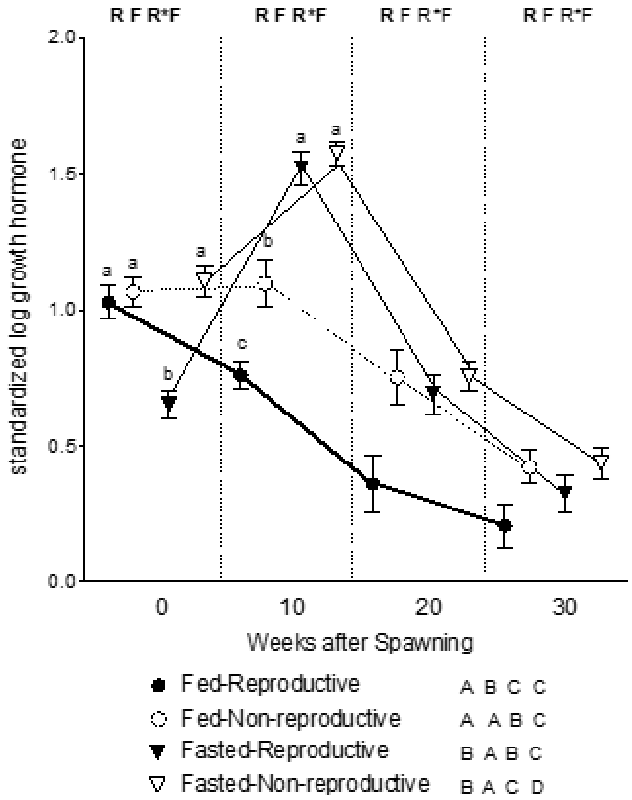


*Figure 2. 13. Growth Hormone in female steelhead trout sampled at 10-week intervals starting at first spawning. Analysis included Fed-Reproductive and Fed-Non-reproductive (closed and open circles, n = 31, 35) and Fasted-Reproductive and Fasted-Non-reproductive (closed and open triangles, n = 24, 44). Symbols indicate mean and bars indicate SEM. Above each sampling point, bolded letters indicate significance (R = Reproductive status, F = Fasting Treatment, R*F = Interaction effect). Rows of letters below the figure indicate significant differences over time within each treatment group. At each sampling point with a significant interaction effect, symbols sharing the same letter do not differ significantly. From Jenkins et al. In Preparation.*

What is the homing fidelity of reconditioned kelts?

To investigate repeat homing fidelity in artificially reconditioned steelhead kelts, we split our investigations into a larger sub-basin level homing fidelity and a much smaller sub-population level homing fidelity. For sub-basin level homing fidelity, we are using the Yakima River as our test population to investigate if post spawn steelhead kelts that are captured emigrating out of the Yakima River, reconditioned, released, then return to the Yakima River basin to spawn a second time. The Yakima River steelhead population consists of four genetically unique major population groups (MPG): the Upper Yakima River, encompassing the mainstem and all tributaries above the confluence with the Naches River; the Naches River system, including Ahtanum Creek and Yakima River mainstem from the confluence of the Naches River down to Toppenish Creek; Toppenish Creek; and Satus Creek (Loxterman and Young 2003). To examine homing fidelity on the sub-population level we investigated the same parameters on a smaller scale using Satus Creek, Toppenish Creek, Ahtanum Creek, Taneum Creek, above Roza Dam (Upper Yakima population) in the Yakima River Basin, and Omak Creek in the Okanogan River

Basin as our test populations to determine if kelts return to the same locations to spawn a second time.

Sub-basin level homing of reconditioned kelt steelhead

After excluding reconditioned kelts that were unsuitable for measuring homing fidelity (see methods), we ended up with a total of 1,210 fish from 2012-2022 that were used for the sub-basin homing study (Hatch et al. 2020). Out of the reconditioned kelts that met the qualifications, a total of 1,210 individual fish were last detected moving upstream at a Yakima Basin mainstem dam PIT tag array, detected at tributary PIT tag arrays, recaptured at Roza Dam prior to spawning, or were recollected as post spawned kelts at the Chandler Juvenile Collection Facility the following emigration season. Only 2 reconditioned kelts had final PIT detections in another sub-basin outside of the Yakima River during this nine-year period. One fish had a final detection on 4/16/2014 in the Walla Walla River and the other was detected in Asotin Creek (tributary of the Snake River) on 4/9/2020. At the sub-basin level this equates to an extremely low stray rate of 0.2%. As noted in Table (2.10) below, we did have an additional 3 fish with final detections at Ice Harbor, Little Goose, and Lower Granite Dams on the lower Snake River during March or April. Although these reconditioned kelts could not be assigned to a specific spawning tributary to be classified as strays at the basin scale, their inclusion in our analysis would increase the stray rate of reconditioned kelts to only 0.4%.

Sub-population level homing of reconditioned kelt steelhead

To investigate homing fidelity at the sub-population level, a total of 161 reconditioned kelts met the qualifications for this analysis (see methods). Each of these fish had sub-population maiden spawner detections and subsequent detections post reconditioning. Maiden spawner origin was determined through detections of first-time spawners outfitted with PIT tags at tributary level or sub-population detection arrays, kelt steelhead collected at a tributary weir, or through parentage analysis of progeny collected in tributaries that confirmed maiden spawning locations. In the Yakima River, all 65 previously PIT tagged fish that were detected as a maiden spawner and reconditioned kelts returned to spawn in the same tributary as their original detection or were detected again at Roza Dam (Upper Yakima population). We have found no evidence of straying in this group of fish.

We also used an ongoing reproductive success study in Satus and Toppenish Creeks to assign Age-0 juveniles to fish later taken into the reconditioning program (Hatch et al. 2020). These parentage results were used as proxy locations for comparison to PIT detections following reconditioning and release from the kelt reconditioning program. A total of 85 fish had both a proxy maiden location (parentage results) and a post reconditioning tributary PIT detection. All fish demonstrated fidelity to their maiden location, with 41 returning to Satus Creek and 44 to Toppenish Creek. No fish were detected at other PIT tag arrays that would have indicated straying behavior.

In Omak Creek (Okanogan River tributary), 11 kelt steelhead were collected at the weir migrating out of the tributary and following reconditioning were released near the mouth of the Okanogan River. All 11 fish were later detected back at the Omak Creek weir on their repeat spawning run. At the sub-population level, we had 100% homing fidelity post reconditioning, as all 146 fish were detected at the same PIT tag arrays or recaptured at the same location as their maiden spawning event (Table 2.10).

Table 2.10. Steelhead homing in the Yakima River and Omak Creek based on PIT detections.

	Sub-Basin Level Homing		**Sub-Population Level Homing**			
Location	Unknown Maiden Tributary		PIT Tag First - Second		Progeny - PIT Second	
	Rehome	Stray	Rehome	Stray	Rehome	Stray
*Yakima R.	1,210	2 (3)	65	0	85	0
Omak Cr.	—	—	11	0	—	—
Totals	1,210	2 (3)	76	0	85	0

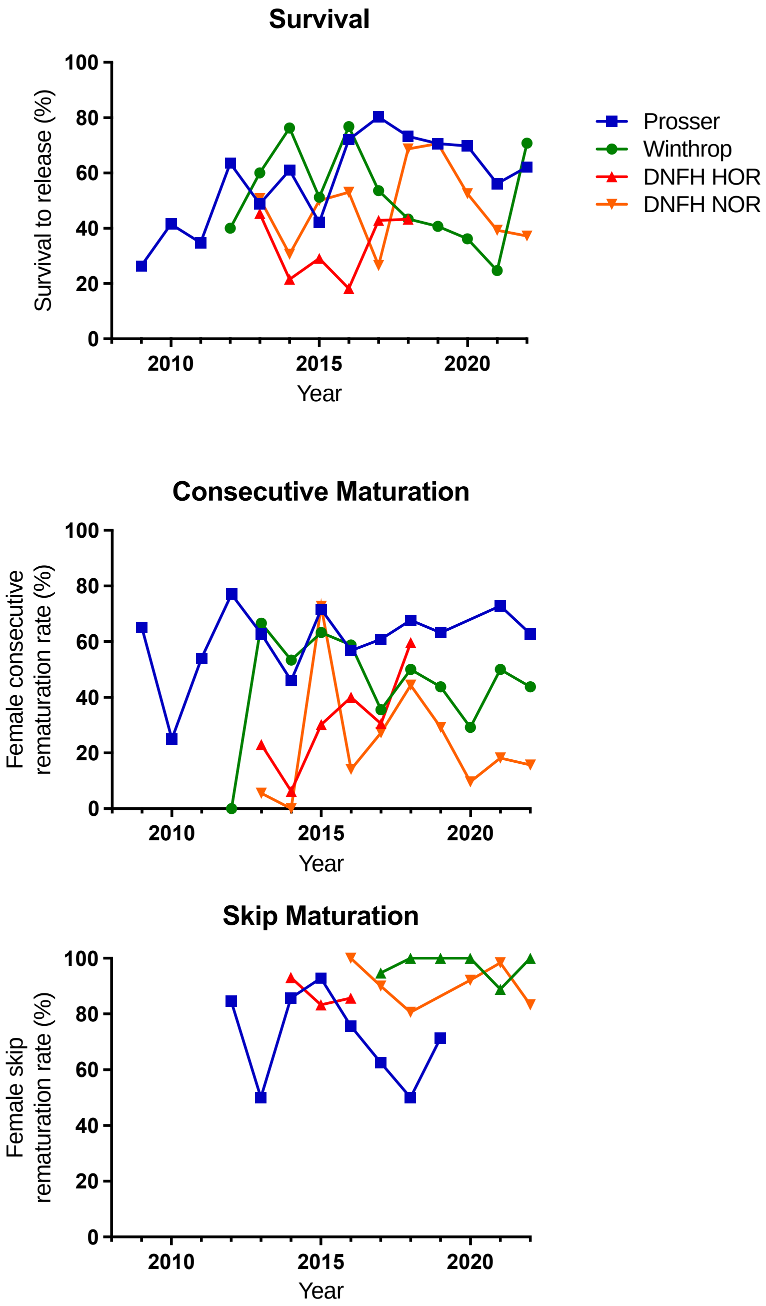
**We did find three reconditioned kelts with final detections at one of the four lower Snake River dams in March/April. These fish were not detected at a sub-basin or sub-population level, but could be considered strays at the basin scale.*

Evaluating Steelhead Kelt Treatments to Increase Iteroparous Spawners in the Columbia River Basin

Geographic Comparison of Reconditioning Programs

Survival and maturation data from Prosser, Winthrop, and Dworshak are shown in Figure 2.14. Survivals in the Prosser project increased beginning in 2012 and have been in the 60-80% range from 2016 to 2022 with one exception (56% in 2021). In 2012, the Prosser project began treating all kelts with emamectin benzoate by intraperitoneal injection for copepod infestation. Previous treatment had been with ivermectin by gavage. We attribute the increased survival to the change to a less toxic treatment. The performance of the Prosser project over the past 7 years has been exceptional and is a tribute to the quality of the fish care in this project. The Prosser project also has the advantage of collecting fish at the reconditioning location, whereas fish must be transported from the collection location to the reconditioning location in other projects. Survival of kelts collected at Lower Granite Dam increased to levels similar to the Prosser project in 2018 and 2019 (not including the mortality event at NPTH in 2019), suggesting that survival rates similar to that found in the Prosser project may be achievable with the Snake River fish. Survival of fish in the Snake River project decreased in 2021-2022, which can be attributed to poorer overall fish condition. Both low run size and changes in the spill regime at Lower Granite Dam in recent years has resulted in fewer fish available for collection, and consequently collection criteria have been adjusted to include fish in poorer condition. Survivals of DNFH hatchery fish were somewhat lower than found for wild origin fish, which may be due to the effects of anesthesia and manual spawning at the hatchery. Further, hatchery returning steelhead have been lethally spawned at DNFH since the hatchery was established in the 1970s, which could have resulted in selection against iteroparity. Survival increased dramatically from 2021 to 2022 at Winthrop. This project returned to copepod treatment by intraperitoneal injection of emamectin benzoate in 2022. Previously, the Winthrop project was not allowed to use this method due to concerns from fish health personnel and suffered heavy mortality due to copepod infestation (M. Abrahamse, personal communication). Overall, results suggest that survivals above 50% are attainable in CRB kelt reconditioning, even in inland populations with a long migration, provided that fish in good condition are available for collection.

Fig. 2.14: Survival and female consecutive and skip maturation rates in CRB kelt reconditioning projects. Snake River fish include natural origin kelts collected at Lower Granite Dam and other sites (DNFH NOR) and air spawned hatchery origin kelts from the DNFH stock (DHFH HOR). Maturation data for skip spawners is from non-mature fish from the previous season held over for an additional year. Prosser 2020 consecutive maturation rates are not shown, because maturation was not assessed by plasma E2 level.



With a few exceptions, consecutive rematuration rates in the Prosser project have consistently been near 60%. Maturation rates for Snake River and Upper Columbia fish have generally been lower, and overall the ranking over the past few years has been Prosser > Upper Columbia > Snake River, which is consistent with the hypothesis that fish with a longer and energetically more demanding migration tend to repeat spawn as skip spawners (Keefer, et al. 2008). The consecutive maturation rate for 2020 at Prosser is not shown because fish were not blood sampled due to the COVID-19 pandemic. Based on visual appearance, the 2020 Prosser maturation rate was 83.8%, but this should not be compared to other maturation rates as it is not based on plasma estradiol level. Some of the variation in maturation rates is attributable to conditions during reconditioning. For example, 2010 was a substantially higher collection year at Prosser than typical, resulting in high densities during reconditioning (Hatch, et al. 2013), Winthrop fish were not given effective treatment for copepods in 2012, and there were issues with fish care on the Snake River project in 2014, 2016, and 2017. The pattern of low consecutive maturation rates in these years suggests that consecutive maturation rate is sensitive to husbandry conditions. However, there were no issues with fish care at Winthrop or in the Snake River in 2020-2022, and yet consecutive maturation rates decreased in these years at both sites relative to previous levels. This can be attributed to the effects of pre-capture environmental conditions, and the collection of fish in poorer condition likely also played a role. Both physiological condition at spawning and post-spawning nutrition influence the consecutive spawning decision in female steelhead kelts (Jenkins, et al. 2023). The variation in consecutive maturation rate from year to year means that projects either need to be able to accommodate varying numbers of skip spawners, or alternative strategies such as transporting and releasing skip spawners need to be explored.

Skip maturation rates in most CRB kelt reconditioning projects have been uniformly high, ranging from 80 to 100%. Surprisingly, however, skip maturation rates at Prosser, the most well established and longest running of the reconditioning projects, have generally been lower than in the other projects. The reasons for this difference are not known, but it may relate to water quality during the winter at the different locations. In other projects, skip maturation rates have been high even in years with a low consecutive maturation rate, such as 2014 and 2016 in the Snake River project or 2017 in the Winthrop project. These results indicate that most kelts that are not rematuring after one summer of reconditioning will mature as skip spawners the next year. In addition, skip spawners have larger eggs and are more fecund than maiden or consecutive spawners, and have greater energy reserves at release (Jenkins, et al. 2018). Thus, skip spawners provide a source of steelhead spawners to seed habitat in years when the numbers of maiden spawners or survival and consecutive maturation rates of reconditioned kelts are low. Given the critically low number of steelhead spawners throughout the CRB in recent years, this is a resource worth supporting.

Chapter 3: Kelt Reconditioning Physiology Studies

Introduction

Analysis and writeup of studies applying methods from fish physiology and endocrinology to issues in kelt reconditioning were continued in 2022. These studies aimed to achieve a sufficiently detailed understanding of the physiology of reconditioning in kelt steelhead to provide a scientific basis for maximizing the success of reconditioning programs. The main ongoing work in this area is screening of kelts for maturation status using plasma estradiol levels, which has become an essential part of the project. In 2022, we sampled blood during the fall at DNFH and Prosser, and provided maturation status of individual fish at DNFH, Prosser, and Winthrop to project managers so that consecutive and skip spawners could be managed appropriately (Section 3.1). Results from 2021 and 2022 were added to a comparison of the performance of the three Columbia River Basin kelt projects in terms of survival and maturation rates (See Chapter 5). We published a fourth study using hatchery origin kelts at Dworshak National fish hatchery (Appendix B) (Jenkins, et al. 2023). This study provides insight into the roles of physiological condition at spawning and post-spawning nutrition on the consecutive spawning decision and builds on our previous work (Jenkins, et al. 2020; Jenkins, et al. 2018; Jenkins, et al. 2019; Medeiros, et al. 2020). We continued analysis and writeup of a study on the growth and reproductive endocrine systems during recovery from spawning in rainbow trout (Section 3.2), which suggests that there is an 8-week process of recovery from spawning that must occur before resources can be allocated to growth.

Chapter 3.1: Reproductive status of wild kelt steelhead

Introduction

An understanding of the reproductive status of female kelt steelhead during reconditioning and at release is required to maximize the success of Columbia River Basin kelt reconditioning projects. Natural steelhead production is limited by the number of female spawners. In order to contribute to ESA-listed steelhead populations, female kelts must not only survive reconditioning but also remature and produce viable eggs. Questions regarding reproductive performance of reconditioned fish underlie issues raised regarding kelt reconditioning projects during ISRP review (ISRP 2011). We believe these issues can be best addressed by research aimed at an improved understanding the life history and physiology of post-spawning steelhead.

Iteroparous female salmonids have two major post-spawning life history trajectories (Chaput and Jones 2006; Keefer, et al. 2008; Rideout, et al. 2005; Rideout and Tomkiewicz 2011). After a spawning event, some fish are able to restore energy lost during migration and spawning, redevelop a mature ovary, and spawn the next year. These fish are termed consecutive spawners. Other fish do not initiate redevelopment of the ovary for the next spawning season, but instead skip a year. These fish are termed skip spawners. We hypothesize that these life history trajectories are the result of the effect of energy balance on maturation decisions made during seasonally defined critical periods. The influential critical period model of the first reproductive maturation (puberty) in salmonids posits that maturation is initiated during a decision window approximately one year prior to spawning (Campbell, et al. 2006; Satterthwaite, et al. 2009; Shearer and Swanson 2000; Thorpe 2007). This decision is made based on energy reserves. If maturation is initiated during this critical period, it may be arrested at a second critical period before the onset of exogenous vitellogenesis, if energy reserves are not sufficient (Yamamoto, et al. 2011). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013; Caldwell, et al. 2014). In post-spawning fish, energy driven decisions take place in the context of the extreme energy deficit incurred by migration and spawning (Penney and Moffitt 2014a, b, 2015). Threshold energy levels for maturation or rematuration are determined by the genetic makeup of the fish and subject to selection (Carlson and Seamons 2008; Hutchings 2011).

Studies conducted in 2009-2011 established that blood levels of estradiol and vitellogenin diverge between rematuring and non-rematuring fish during reconditioning. Estradiol is the principal female gonadal steroid in fishes, which regulates many aspects of reproductive development, and vitellogenin is a phospholipoprotein produced by the liver under regulation by estradiol which provides most of the material for ovarian development. Estradiol indicates maturation earlier than vitellogenin, and the cost of the estradiol assay is about 1/4th of the cost of the vitellogenin assay.

During 2022, we measured estradiol level in a large number of blood samples. We collected blood from fish in the reconditioning programs at Dworshak National Fish Hatchery (DNFH) and Yakama Nation Hatchery (Prosser, WA), ran plasma estradiol assays, and provided maturation status to project managers so that rematuring fish could be released and non-rematuring fish retained for further reconditioning (at DNFH). Additionally, we collaborated with colleagues in the Upper Columbia reconditioning project at Winthrop National Fish Hatchery (WNFH) to measure estradiol levels in samples they collected from their reconditioned kelts.

Methods

Fish Collection and Husbandry

Steelhead kelts were collected and reconditioned at Prosser Hatchery (Washington), Dworshak National Fish Hatchery (DNFH; Idaho), and Winthrop National Fish Hatchery (WNFH; Washington) as described elsewhere (Abrahamse and Murdoch 2013, 2014) (Table 3.1).

Table 3. 1. Wild steelhead kelts held for long-term reconditioning for the 2021 reconditioning year. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery.

Location	Fish type	Total # Fish	Collected in 2022	Collected in 2021	Notes
Prosser	Wild kelts	118	118	N/A	Very low collection rate for 2022
DNFH	Wild kelts	201	183	18	Low collection rate for 2022
WNFH	Wild kelts	72	66	6	

Sampling

Fish were blood sampled on the indicated dates (Table 3.2). During blood sampling, blood (approximately 2 mL) was drawn from the caudal vein using heparinized syringes (ammonium heparin, 10 mg/ml) and centrifuged (5 min, 5000 x g). Plasma was collected and frozen on dry ice in the field prior to storage at -80°C. In addition to blood sampling, the length, weight and sex of fish were recorded, and a reading of muscle lipid levels was taken with a Distell Fish Fatmeter (Distell Inc., West Lothian, Scotland), using the rainbow trout muscle lipid setting (Trout-1) at the two most anterior measurement sites recommended by the manufacturer (Colt and Shearer 2001; Crossin and Hinch 2005).

Table 3. 2. Wild steelhead kelts sampled during the fall of 2021. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery.

Location	Sample date	Fish type	# Fish	Notes
Prosser	10/4/22	Wild kelts	82	All fish were collected in 2022
DNFH	10/10/22	Wild kelts	82	Fish were collected in 2021 (n = 12) and 2022 (n = 70)
WNFH	9/21/22	Wild kelts	53	Fish were collected in 2021 (n = 5) and 2021 (n = 48)

Estradiol Assay

Fish plasma level of estradiol-17β (E2) is an indicator of reproductive development. Fish plasma samples must be solvent extracted prior to E2 assay to remove interfering substances. Plasma samples (250 µL) were extracted twice consecutively in 10 mL glass tubes with anhydrous diethyl ether (JT Baker, Avantor Performance Materials, Inc.; Center Valley, PA). 2.0 mL diethyl ether was added to each tube and samples were vortexed for 1 m, the phases allowed to separate for 7 minutes, and then frozen on dry ice. After 5 minutes, the aqueous phase was inspected to ensure that it was frozen solid, and the solvent fraction was then poured off into a 5 mL glass tube. Diethyl ether extracts were then placed in a 54°C water bath (OA-SYS™Heating System; Organomation Associates, Inc; Berlin, MA) and dried down under a gentle stream of N_2 directed *via* a nitrogen evaporator manifold (N-EVAP™ 112; Organomation Associates, Inc; Berlin, MA). A second extraction of the remaining aqueous fraction from each plasma sample was then performed as described above and was pooled with the first extract. Dried extracts of fish plasma were resuspended in 250 µL assay buffer from the estradiol assay kit.
Plasma E2 concentrations were assayed by an enzyme immunoassay using an acetylcholinesterase linked estradiol tracer (Cayman Chemical; Ann Arbor, MI). Extracted plasma samples were appropriately diluted and duplicate technical replicates assayed according to the manufacturer's instruction manual provided with the kit.

Results

Plasma E2 levels were bimodally distributed in blood samples taken from female kelts in all projects at a pre-release sampling in the fall (Figs 3.1, 3.2, 3.3). As found in previous years, the division between the lower and higher modes was approximately 1000 pg/ml E2 at all locations. However, fish with E2 levels close to 1000 pg/ml that appear to group with the lower mode could represent a group of fish maturing more slowly than the rest of the upper mode. To err on the side of caution and ensure all maturing fish are released, a borderline category of 600-1000 pg/mL is included and fish in this category were released. As is typical, the rematuration rate of female kelts as consecutive spawners in 2022 was highest at Prosser - females rematured at a rate of 66.7%. Consecutive spawners from other programs on the Snake River and Upper

Columbia River had lower rates of rematuration for 2022, with only 15.7% of the Snake River fish rematuring and 43.8% of the Upper Columbia River fish rematuring. As with previous years, the rematuration rate of female kelts held for a second year of reconditioning was higher than consecutive spawners for the Snake River fish (83.3%) as well as the Upper Columbia River fish (100%).

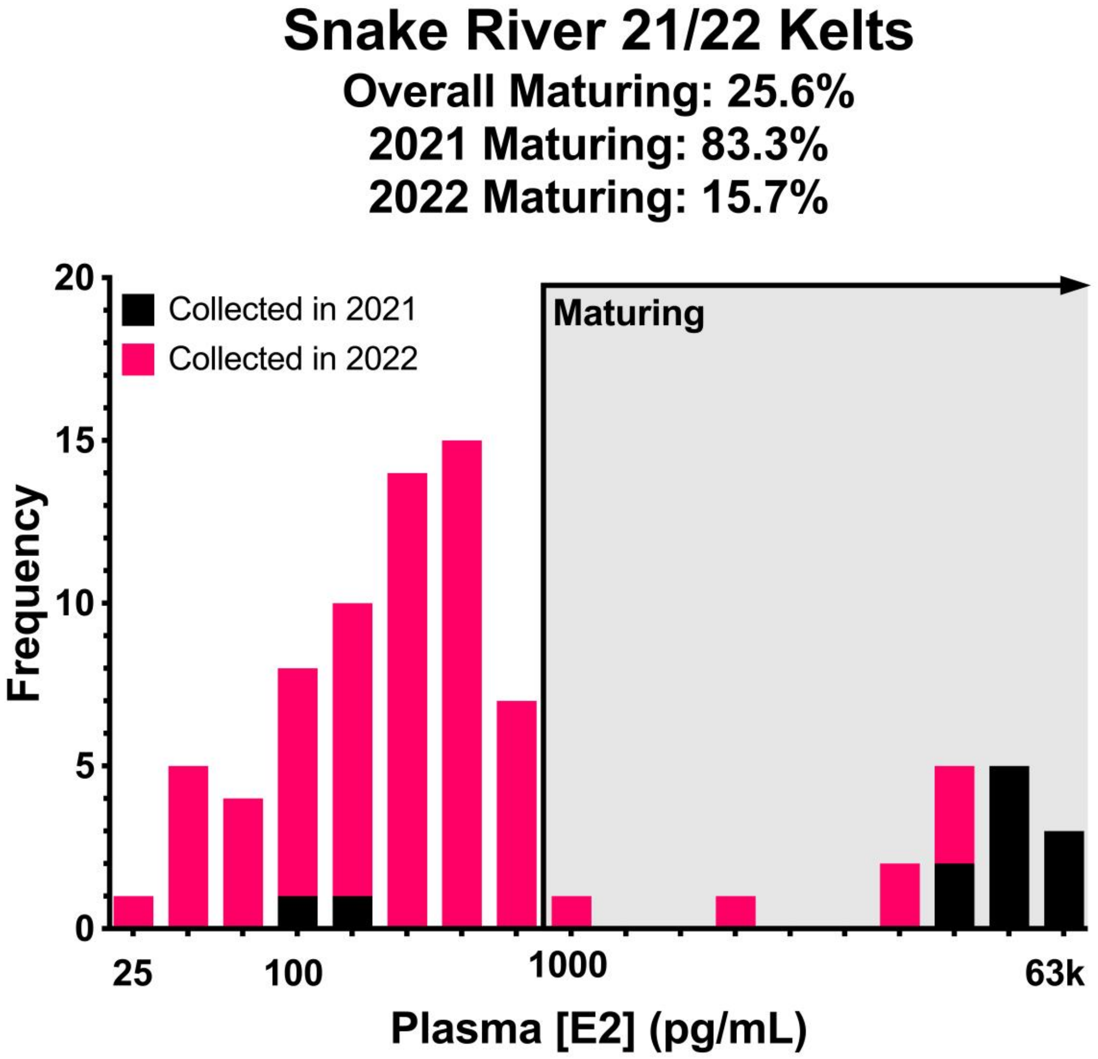


Figure 3. 1. Plasma estradiol (E2) levels in wild female Snake River kelts sampled in fall of 2022.

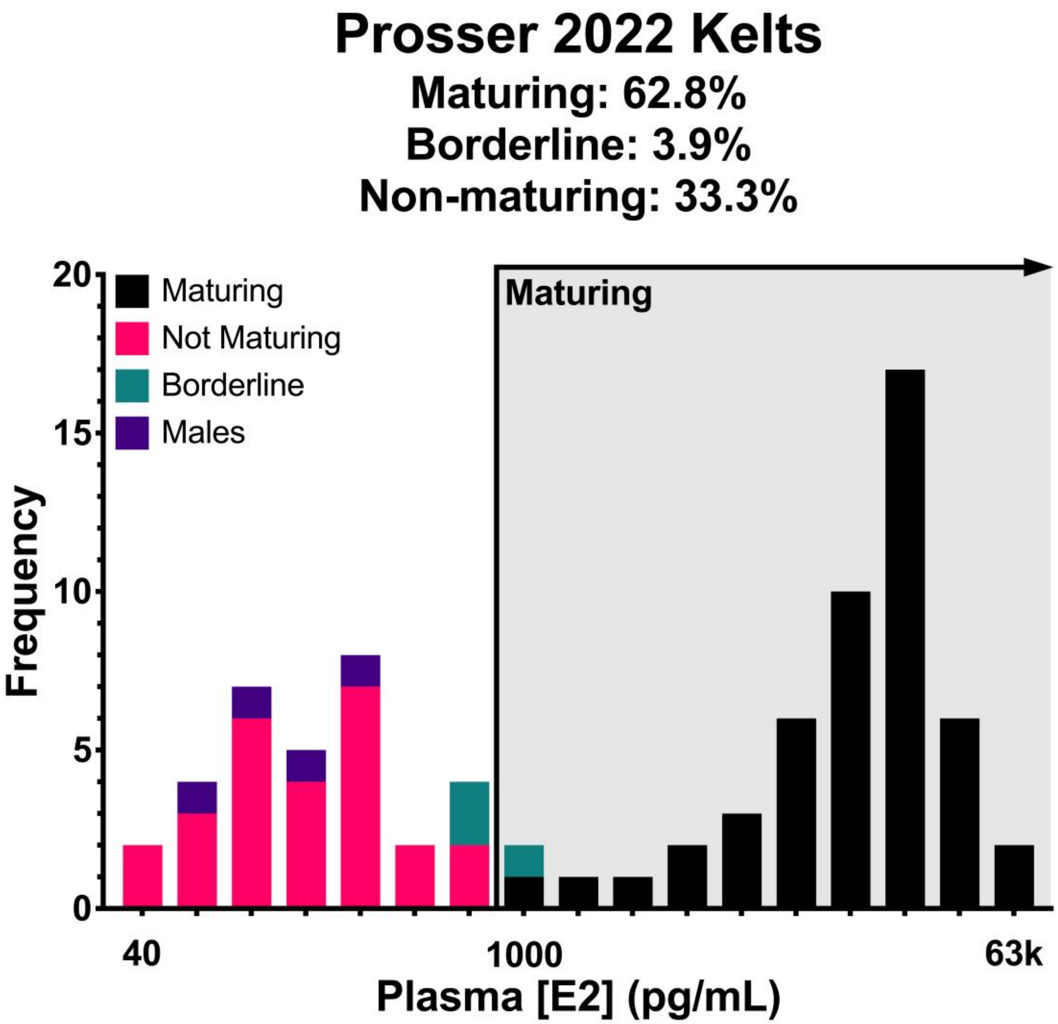


Figure 3. 2: Plasma estradiol (E2) levels in wild female Yakima River kelts sampled in fall of 2022.

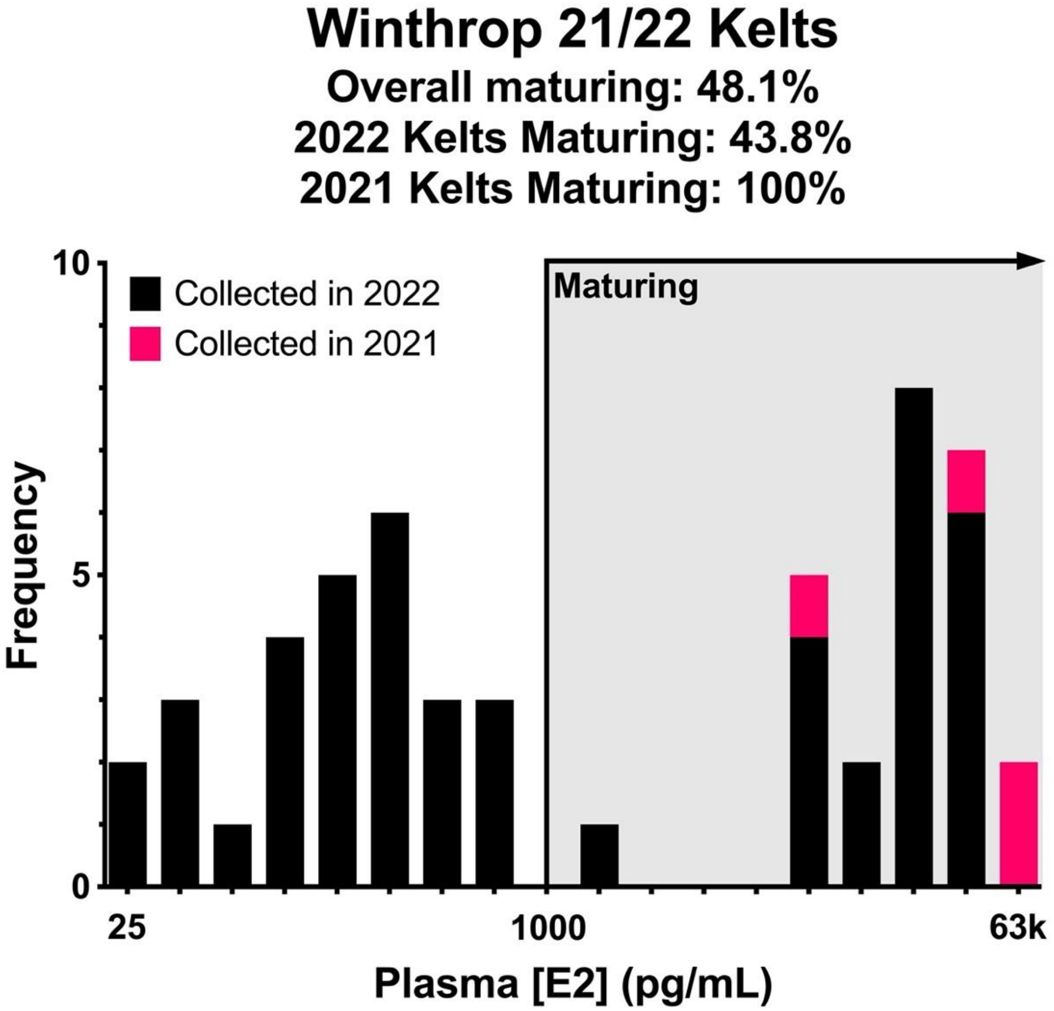


Figure 3. 3:Plasma estradiol (E2) levels in wild female Upper Columbia River kelts sampled in fall of 2022.

Due to the relatively low survival rate for hold over fish at Prosser hatchery (averaging approximately 13.7%), all fish are currently being released regardless of maturation status. Thus, there are no skip spawners at Prosser. Skip spawner survival for fish collected in 2021 and held for further reconditioning was on parr or better compared with previous years: 61.1% at DNFH and 83.3% at WNFH. Survival of fish collected in 2022 to release was 62.7% at Prosser, 37.2% at DNFH, and 71.2% at WNFH (Table 3.3).

Table 3. 3: Wild steelhead kelts released during the fall of 2022. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery.

Location	Release date	Fish type	# Fish	Notes
Prosser	11/2/22	Wild kelts	74	All surviving fish collected in 2022
DNFH	11/21/22	Wild kelts	22	Fish were collected in 2021 (n = 11) and 2022 (n = 11)
WNFH	11/9/22	Wild kelts	25	Fish were collected in 2021 (n = 4) and 2022 (n = 21)

Discussion

It is now well established that some female steelhead kelts remature after a summer or more of reconditioning, whereas other fish do not, and that plasma estradiol level from mid-June onward indicates maturation status (Jenkins et al. 2018). Evidence in both steelhead kelts and post-spawning rainbow trout suggests that the initial decision to remature is made early, before mid-July for kelts and during the 10 weeks after spawning in rainbow trout (Bromage, et al. 1992; Caldwell et al. 2013; Caldwell et al. 2014; Hatch, et al. 2013a; Jenkins et al. 2018). At all reconditioning sites, plasma estradiol levels in rematuring and non-rematuring kelts for 2022 were similar to previous years and were similar to those seen in other projects.

Female consecutive maturation rates were variable among the projects this season. It is possible that this relates to pre-capture environmental conditions. In previous years, the relatively low consecutive maturation rates found in Snake River kelts has been in line with what has been observed previously in Snake River steelhead, and steelhead from the Skeena and Nass systems in British Columbia, which have a life history similar to Snake River B-run steelhead. These cohorts have been found to repeat spawn predominantly as skip spawners (Chudyk 1976; Keefer et al. 2008; Moore, et al. 1995). This has been hypothesized to be due to the longer migration and later spawn timing of these fish. With only 30% of fish rematuring after a year of reconditioning (on average), consecutive rematuration is observed in less than half of this population, implying that pre-capture environmental conditions may dictate the reproductive strategy employed. This could be the result of the warmer water temperatures the Columbia River Basin has been experiencing the past couple of summers, requiring a longer recovery period before the kelts are able to mature again (even with reconditioning). This is supported by the consistently high rates of maturation in the fish held for a second year of reconditioning. The higher mortality at WNFH and DNFH observed in 2021, coupled with the lower-than-average rate of consecutive rematuration for that year, provides support for the theory that pre-capture environmental conditions are an important factor when evaluating what contributes to rematuration. In addition, fish were not given effective treatment for parasitic copepods at WNFH in 2021 due to concerns about our method for treatment (M. Abrahamse, personal communication). The rates of maturation in this year's skip spawners (i.e., fish collected in 2021) were on par with previous years, providing further evidence that the reconditioning program is perhaps acting as an (artificial) refuge for life histories that would otherwise be in decline. This is especially important during recent years when adult returns have been well below the historic average. Of note, at the 2022 Annual AFS meeting (held in Spokane, WA), Tim Copeland of Idaho's Department of Fish and Game discussed his belief that post-spawning nutrition availability plays an important role in recovery, and thus repeat spawning. During his presentation he expressed his support for the reconditioning program and the role it appears to be playing in keeping the Snake River steelhead population alive.

Non-rematuring fish collected from the Snake River and Upper Columbia held for a second year rematured at very high rates (83.3% or higher) in 2022. This adds to a growing body of data showing that non-rematuring females will remature as skip spawners if held for a second year. Skip spawning is a natural life history in Columbia Basin steelhead. Increased size, fecundity, and energy reserves in skip spawners result in greater relative reproductive success versus maidens or consecutive repeat spawners (Jenkins et al. 2018). The presence of skip spawners increases life history diversity, which would be expected to increase population stability in steelhead populations (Moore, et al. 2014; Schindler, et al. 2010). Moreover, whether and how much culture conditions can influence the proportion of consecutive and skip spawning kelts in captive reconditioning is not well understood. These considerations suggest that Columbia Basin kelt reconditioning programs should find ways to accommodate the skip spawner life history, a fact that's becoming increasingly important as run numbers show an overall decrease and the site's survival rate increases. Together, these data point out the important role the program is playing in keeping the local population healthy and diverse.

Hold over survival in Snake River fish is relatively high (typically over 60%), which is in contrast to the low survival experienced by fish held for an additional year at Prosser. The difference could be due to population-specific life history differences but could also be due to over winter water quality. The Prosser facility is located in an area that experiences heavy runoff. Considering that the low hold over survival has occurred since the site began holding non-maturing fish in 2016, the suitability of Prosser as a hold over reconditioning facility was re-evaluated. Considering the continued low returns, it was decided that all fish would once again be returned to the river, regardless of maturation status. More suitable hold over sites will be discussed in 2023.

Typically, there is high mortality in recently collected fish; however, DNFH has experienced higher mortality than is typically observed for the second year in a row. Survival of recently collected fish at all locations tends to average around 60%, whereas survival at DNFH was only 37.2% this year and 39.3% last year. As with overall collection numbers being far less than is typical, the trend for decreased survival is most likely the result of the poor ocean conditions and elevated river water temperatures (i.e., climate change) during their return migration. Compounding the issue is that females appear to be more affected by the worsening conditions (Hatch, et al. 2013b; Keefer et al. 2008), making the reconditioning program more likely to be negatively impacted by this effect. In addition, fewer kelts are available for collection at the Lower Granite Dam juvenile bypass because of changes in the spill regime. This results in fish in poorer condition being collected for reconditioning. To counteract the effects of climate change and declining return numbers, it seems prudent to increase the number of collection sites and investigate ways in which collection efforts could be improved (e.g., better holding conditions, improved sorting and transportation that decrease stress levels, discussing how changes to spill patterns could help collection numbers).

Section 3.2: Progress Report: Effects of post-spawning ration restriction on reproductive development and the growth hormone/insulin-like growth factor-1 axis in female rainbow trout (*Oncorhynchus mykiss*)

The post-spawning period is critical in the survival and reproductive life history trajectory of steelhead kelts. Energy reserves are at a low level due to the demands of migration, gonadal development, and spawning (Penney and Moffitt 2014b, 2015). Post-spawning fish are in a profoundly catabolic state with elevated cortisol resulting in immunosuppression and breakdown of tissues to support metabolism, which results in programmed post-spawning mortality in the semelparous salmonids, and high mortality in the iteroparous anadromous species such as steelhead and Atlantic salmon (Barry, et al. 2010; Barry, et al. 2001; Dickhoff 1989; Mommsen 2004; Mommsen, et al. 1999). The gut is atrophied due to prolonged fasting, and gut function must be restored to access energy from feeding, which requires energy (Krogdahl and Bakke-McKellep 2005; Penney and Moffitt 2014a; Simpkins, et al. 2003; Zaldua and Naya 2014). Due to physiological constraints on gonadal development, fish must begin investment of energy into gonadal recrudescence soon after spawning if they are to follow a consecutive repeat spawning life history schedule. However, little is known about the physiology of the post-spawning period. To fill in this knowledge gap, we previously established post-spawning female rainbow trout as an experimental model to supplement studies using steelhead kelts (Caldwell et al. 2013; Caldwell et al. 2014). During 2017, we conducted a follow-on study using the rainbow trout model. For this study, we used newly developed assays for growth hormone and insulin-like growth factor-1, key hormones in the endocrine system that regulates growth (Medeiros et al. 2020), to examine the response of the growth and reproductive endocrine systems during recovery from spawning and nutritional restriction. During 2022, we continued analysis and interpretation of the results from this study, and preparation of a manuscript for submission to a scientific journal.

Chapter 4. Building a Snake River Kelt Reconditioning Facility

Background

In the Columbia Basin Fish Accord Agreement that CRITFC is party to, $2M was included for capital construction of a Snake River Kelt Reconditioning Facility. The Northwest Power and Conservation Council (NWPCC) three-step review process is triggered for any artificial production initiative that involves the construction of new production facilities. In 2016, we drafted a Master Plan, reviewed the plan with co-managers and action agencies and submitted it the NWPCC for review by the Independent Science Review Panel (ISRP). In December 2016, the NWPCC accepted our Master Plan and recommended that we proceed to final design of the facility. This Master Plan would result in the fabrication of new facilities at an existing propagation facility.

The initial review of the Master Plan by the ISRP was completed in May 2016. The ISRP response is summary was:

"The Master Plan is well written and contains an excellent summary of the extensive steelhead reconditioning work that has occurred in the Basin. Moreover, we compliment the proponents for investigating and addressing the many difficulties associated with steelhead reconditioning. Numerous challenges associated with fish culture had to be addressed, including establishing appropriate holding and rearing environments, formulating diets, and developing disease control protocols. The effects of long-term reconditioning on gamete viability, fidelity to natal streams, and ability to reproduce in nature were investigated. Comparisons that evaluated the potential benefits of various kelt treatments that ranged from simple direct transportation past downstream dams to long-term reconditioning lasting from 6 to 20 months were also conducted. In general, the results of these assessments indicated that long-term reconditioning of kelts appears to be a promising approach that might lead to a viable conservation strategy for steelhead.

The proponents acknowledge that the submitted Master Plan does not yet have all the necessary components for a Step 1 review. It currently lacks a Hatchery Genetic Management Plan (HGMP), and work is needed on the program's Research, Monitoring and Evaluation Plan and Comprehensive Environmental Assessment. Before producing these elements of the Master Plan, the proponents requested that the ISRP determine if the program's preferred location for a long-term reconditioning facility, for Snake River B-run steelhead, is appropriate.

More information is needed before a decision about the location of the proposed long-term reconditioning facility can be reached. Specifically, information on the following issues is requested in the updated Step 1 Master Plan. Additional comments provided in the ISRP's full report should also be considered in the revision.

The biological and ecological rationale for annually increasing B-run steelhead escapement by 180 reconditioned female kelts needs to be explained in the Master Plan.
Clarification on why male kelts are not included in the proposed reconditioning program is needed.

The biological escapement goals for B-run steelhead populations in the Snake River subbasin should be in the Master Plan along with a description of what project “success” entails. To what extent, for example, are reconditioned kelts expected to contribute to the rebuilding of natural steelhead populations and eventually to fisheries?

If available, information on the abundance and status and trends of B-run steelhead populations in the Clearwater and Salmon River subbasins should be provided in the Master Plan. Current spawning levels of B-run steelhead in the Snake River Basin should also be described with reference to numerical objectives for natural spawning steelhead. Additionally, a brief overview of the factors limiting each of these populations should be added to the Plan. Substantial hatchery and habitat restoration actions affecting B-run steelhead are occurring in the Snake River subbasin. The Master Plan should briefly describe these programs and indicate how the proponent’s goal of annually releasing 180 reconditioned kelts will be coordinated with ongoing habitat restoration and existing hatchery programs.

As it is currently designed, the kelt reconditioning program will recondition female B-run steelhead kelts without targeting specific populations. It would seem that capturing, reconditioning, and releasing kelts from populations that have the potential to accommodate additional spawners would be a more efficient and productive way of directing this strategy. The Master Plan should explain why a more focused program was not considered.
The Master Plan should discuss the infrastructural needs of a more focused and integrated reconditioning program. If the project, for instance, were to narrow its focus on B-run populations that could benefit from the addition of reconditioned kelts, would facilities at Dworshak National Fish Hatchery be adequate to meet these new escapement objectives?
The Master Plan should compare the benefits and drawbacks of increasing B-run steelhead escapements by modifying harvest regulations, by long-term reconditioning for adult release, and long-term reconditioning for captive breeding and smolt release.

Some discussion of the genetic risks that may accompany reconditioning (e.g., heritable epigenetic effects and domestication selection) needs to be added to the Master Plan or incorporated into the Plan’s HGMP.”

We revised the Master Plan and submitted the document to the ISRP in July 2016 and received “meets scientific review criteria (qualified)” recommendation on September 27, 2016.

At the November 2016 NWPCC meeting in Coeur d’ Alene, we presented our Master Plan to the Council’s Fish Committee. The Fish Committee received the plan favorably and recommended that it be presented to the full Council in December. At the December Council meeting we again presented the Master Plan and received a recommendation from the Council to proceed to the Final Design stage of the 3-step process.

In 2017, advancements were made in drafting a Monitoring and Evaluation (M&E) Plan and environmental compliance documents. Also, in 2017, we met with BPA and determined that BPA would solicit through a Request for Proposals (RFP) for a firm to design and build the kelt facility. The pace of this action has been slow and there are several components that must be completed. These include a Memorandum of Understanding (MOU) for construction, Operation and Maintenance (O&M) funding plans, Facility Designs, completion of the Northwest Power and Planning Council's Step 3, and construction of the facility.

In 2019, discussions between CRITFC and BPA continued regarding soliciting for a design and construction firm and development of a M&E and O&M plan. Bonneville Power Administration solicited proposals for the design phase in November 2019. A review group was formed to select a design firm and received completed designs at the end of 2020.

2022 Progress

BPA released an RFP for construction of the Snake River Kelt Facility in the Spring of 2022. Only a single contractor responded to the RFP and the review team decided to postpone the project until 2023 in hopes of receiving more bids and a lower cost. We anticipate releasing an RFP early in 2023. CRITFC has identified three additional contractors that are interested in bidding on the project. This additional competition should result in more competitive bids. Construction should be completed by the fall of 2024.

Chapter 5: Project wrap up, delineation of necessary follow up monitoring and research, complete manuscripts, transition to other locations or uses of kelt reconditioning technology.

The ISRP has identified 3 major points that this project should focus on before moving towards full-scale basin wide production:

1. Focus on identifying at-risk populations for collection utilizing genetic tools to focus reconditioning efforts on these populations specifically. This task was accomplished in 2021 please see Hatch et al 2021 for further details.
2. Transferring kelt reconditioning knowledge and techniques to other at-risk populations in the Columbia River Basin to further assist in recovery efforts. This task was accomplished in 2021 please see Hatch et al 2021 for further details.
3. Development of a plan to provide the documented kelt technological knowledge to other specific agencies for implementation. We intend to have this component completed by the 2023 Annual Report. Details gleamed from the following listed below will be used to complete this portion of the ISRP request.

Adaptive Management & Lessons Learned

1. Columbia River steelhead populations upstream of Bonneville Dam are listed under ESA and need novel recovery strategies.
2. There is a relatively large abundance of kelt steelhead in the Columbia River Basin even in the upper most areas.
3. In general, repeat spawning steelhead make up a very small proportion of the spawning run.
4. Increasing repeat spawners in steelhead populations can have many positive effects on populations including increasing; genetic diversity, lifetime fecundity, and fitness since genes are distributed across generations.
5. Long-term reconditioning kelt steelhead provides 5 to over 100 times more repeat spawners than leaving the fish in the river.
6. Physiology studies have provided us with a much better understanding of energetic and physiological status of kelts, improved our understanding of alternative life histories in post-spawning fish, and improved survival and health of reconditioned fish.
7. Blood hormone assays are useful to classify consecutive and skip spawner steelhead. Future work needs to focus on optimizing strategies for skip spawner contributions.
8. There appears to be a reduction in the B-run steelhead composition between the maiden and kelt stage, but the B-run composition of repeat spawners is similar to the kelt composition. Underlying biological and behavioral factors contributing to such discrepancies are not well understood but likely warrant further investigation of potential causes. With more data

including escapement comparisons, it may be possible to refine the confidence in estimated rates of iteroparity among Reporting Group's (RG's).

9. Age appears to be less of a factor in rates of iteroparity than size. While the A-run life history was observed to be present among all reporting groups, so too were the B-run life history.
10. Despite the understanding in recent years that the B-run life history is relatively uncommon outside the middle and south forks of both the Clearwater River and Salmon River, our results suggest otherwise. In fact, age 2-ocean fish were dominant among all 10 reporting groups. This finding has implications for management of steelhead populations in the basin and provides evidence that regionally based classifications of life history types or their distributions warrants reconsideration.
11. The upper Salmon River region produces a disproportionate number of Snake River kelt steelhead and is presumably an important factor in spawner abundance for that region. This result is mirrored among hatchery-origin fish.
12. Adding a production level kelt reconditioning facility at Nez Perce Tribal Hatchery will make achieving the goal RPA 33 possible, i.e., increase the abundance on adult B-run steelhead by 6%.
13. The Snake River Kelt Reconditioning Facility Master Plan was submitted and favorably review by the ISRP and recommended to proceed to final design by the NWPCC in December of 2016.
14. Reproductive success studies are underway at a variety of scales: hatchery analog, spawning channel, and natural river. Results are positive.
15. Artificially reconditioned kelt steelhead appear to repeat home with high fidelity. Data indicates that natural repeat spawners in the Snake River exhibited a 15% stray rate.
16. Concluded with the Cle Elum spawning channel.
17. Kelt biophysiological decision to remature is made soon after spawning.
18. As a result of this project 1,700 kelt steelhead were collected in the Snake River since 2012 and 697 of those fish were reconditioned and released back into the Snake River.
19. As a result of this project 7,868 kelt steelhead were collected in the Yakima River since 2008 and 3,416 of those fish were reconditioned and released back into the Yakima River.
20. Kelt Reconditioning, during years of low steelhead returns, effectively acts as a stop gap or safety net measure that should allow for a larger production of the juvenile population than normal under poor return years. This increase in juvenile production, should rearing and migration conditions improve, would translate into additional adult returns later, thus decreasing the time period for recovery after poor run years.
21. GSI analysis revealed that >50% of the reconditioned kelts released in the Snake are from B-run MPGs (Clearwater and Salmon rivers). These fish are important for meeting the goal of RPA 33. The National Marine Fisheries Service has issued a new Biological Opinion for operation of the Columbia River Hydrosystem (NMFS 2019) and plan to issue supplanting BiOp for the FCRPS in 2020.
22. Conducted and produced valuable kelt research on rematuration of steelhead kelts and how environmental factors play into rematuration and how we may be able to better identify sequential/skip spawners to address management of steelhead kelts in the Columbia River Basin See Jenkins et al 2020 and Medeiros et al 2020.
23. New and improved redundancy systems and protocols are being put in place at Nez Perce Tribal Hatchery to prevent catastrophic loss at the facility until a dedicated facility is constructed, which will have better fail-safe systems in place.

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Appendices

A: Master Kelt Tracking Table

Strategy	Year	Capture Location	# Collected	# released	[redacted]	# @ Bonneville	Return Rate to Bonneville (%)	# @ Lower Granite Dam (or Prosser)	Return Rate to Lower Granite Dam (or Prosser) (%)	Transportation Benefit relative to in-river	1.66	Transportation Benefit relative to Bonneville natural
In-river	2005	Prosser	67	67	[redacted]	3	4.5			1.54	2.70	25.61
In-river	2006	Prosser	51	51	[redacted]	1	2.0			0.67	1.18	3.16
In-river	2007	Prosser	53	53	[redacted]	3	5.7			1.95	3.41	9.28
In-river	2008	Prosser	88	88	[redacted]	4	4.5			1.56	2.74	6.64
In-river	2009	Prosser	58	58	[redacted]	3	5.2			1.78	3.12	11.54
In-river	2010	Prosser	155	154	[redacted]	2	1.3			0.44	0.78	3.74
In-river	2011	Prosser	85	85	[redacted]	3	3.5			1.21	2.13	7.01
In-river	2012	Prosser	59	59	[redacted]	2	3.4			1.17	2.04	3.15
In-river	2013	Prosser	52	52	[redacted]	0	0.0			0.00	0.00	0.00
In-river	2014	Prosser	45	45	[redacted]	3	6.7			2.29	4.02	11.52
In-river	2015	Prosser	121	121	[redacted]	0	0.0	0	0.0	0.00	0.00	0.00
In-river	2016	Prosser	56	56	[redacted]	2	3.6	2	3.6	1.23	2.15	57.50
In-river	2017	Prosser	5	5	[redacted]	0	0.0	0	0.0	0.00	0.00	0.00
Disc. In 2018					[redacted]							
Total and weighted mean	13		***895***	***894***	[redacted]	***26***	***2.91***	***2***	***0.22***	***1.07***	***1.75***	***5.64***
In-river	2009	Lower Granite	178	176	[redacted]	2	1.1	2	1.1	4.67	0.68	1.96
In-river	2010	Lower Granite	1410	1399	[redacted]	5	0.4	4	0.3	1.47	0.21	0.42
In-river	2011	Lower Granite	1633	1613	[redacted]	3	0.2	3	0.2	0.76	0.11	0.10
In-river	2012	Lower Granite	2098	2098	[redacted]	4	0.2	3	0.1	0.79	0.11	0.10
In-river	2013	Lower Granite	840	827	[redacted]	3	0.4	2	0.2	1.48	0.22	0.37
In-river	2014	Lower Granite	2584	2571	[redacted]	11	0.4	9	0.4	1.77	0.26	0.50
In-river	2015	Lower Granite	1195	1193	[redacted]	0	0.0	0	0.0	0.00	0.00	0.00
In-river	2016	Lower Granite	1841	1837	[redacted]	4	0.2	2	0.1	0.90	0.13	2.11
In-river	2017	Lower Granite	824	821	[redacted]	0	0.0	0	0.0	0.00	0.00	0.00
In-river	2018	Lower Granite	868	863	[redacted]	3	0.3	1	0.1	1.44	0.21	0.31
In-river	2019	Lower Granite	1062	1034	[redacted]	0	0.0	0	0.0	0	0.00	0.00
In-river	2020	Lower Granite	296	284	[redacted]	TBD 2023	TBD 2023	TBD 2023	TBD 2023	TBD 2023	TBD 2023	TBD 2023
In-river	2021	Lower Granite	250	248	[redacted]							
Disc. In 2022					[redacted]							
Total and weighted mean	12		***15079***	***14964***	[redacted]	***35***	***0.2***	***26***	***0.2***	***1.21***	***0.15***	***0.28***
In-river	2020	Little Goose	22	22		TBD 2023	TBD 2023	TBD 2023	TBD 2023	TBD 2023	TBD 2023	TBD 2023
In-river	2021	Little Goose	14	14								
Total and weighted mean	2		***36***	***36***								

Strategy	**Year**	**Capture Location**	**# Collected**	**# released**	[redacted]	**# @ Bonneville**	[redacted]	**# @ Prosser**	[redacted]
Transported Immature (Fall Release @ Hamilton Is.)	2019	Prosser	***103***	***103***	[redacted]	***0***	[redacted]	[redacted]	[redacted]
Transported Immature (Fall Release @ Hamilton Is.)	2020	Prosser	***52***	***52***	[redacted]	***0***	[redacted]	[redacted]	[redacted]
Transported Immature (Fall Release @ Hamilton Is.)	2021	Prosser	***53***	***53***	[redacted]	***0***	[redacted]	[redacted]	[redacted]
total years	**3**		***208***	***208***	[redacted]	***0***	[redacted]	[redacted]	[redacted]

Strategy	**Year**	**Capture Location**	**# Collected**	**# released**	**S @ release (%)**	**# remature**	**Retained**	**skip remature**	[redacted]	**Transportation Benefit relative to in-river**	**Treatment benefit relative to Hockersmith 1.66**	**Transportation Benefit relative to Bonneville natural**
Long-term	2000	Prosser	512	91	17.77				[redacted]	NA	10.71	NA
Long-term	2001	Prosser	551	197	35.75				[redacted]	NA	21.54	NA
Long-term	2002	Prosser	420	140	33.33				[redacted]	NA	20.08	NA
Long-term	2003	Prosser	482	298	61.83				[redacted]	NA	37.24	NA
Long-term	2004	Prosser	662	253	38.22				[redacted]	NA	23.02	107.49
Long-term	2005	Prosser	386	86	22.28				[redacted]	4.98	13.42	127.44
Long-term	2006	Prosser	279	85	30.47				[redacted]	15.54	18.35	49.15
Long-term	2007	Prosser	422	221	52.37				[redacted]	9.25	31.55	85.84
Long-term	2008	Prosser	472	269	56.99				[redacted]	12.54	34.33	83.27
Long-term	2009	Prosser	510	140	27.45	91			[redacted]	5.31	16.54	61.24
Long-term	2010	Prosser	1157	404	34.92	101			[redacted]	27.06	21.03	101.26
Long-term	2011	Prosser	680	223	32.79	120			[redacted]	9.29	19.76	65.17
Long-term	2012	Prosser	550	340	61.82	275			[redacted]	18.24	37.24	57.41
Long-term	2013	Prosser	546	266	48.72	166	41	8	[redacted]	16.77	29.35	44.47
Long-term	2014	Prosser	481	292	60.71	149	96	22	[redacted]	9.11	36.57	104.90
Long-term	2015	Prosser	1098	396	36.07	382	74	37	[redacted]	12.41	21.73	69.96
Long-term	2016	Prosser	471	360	76.43	236	74	15	[redacted]	21.40	46.04	1230.57
Long-term	2017	Prosser	118	55	46.61	55	29	4	[redacted]	16.04	28.08	90.42
Long-term	2018	Prosser	227	103	45.37	103	49	5	[redacted]	15.62	27.33	67.76
Long-term	2019	Prosser	371	248	66.85	145	NA	NA	[redacted]	23.01	40.27	182.71
Long-term	2020	Prosser	463	320	69.11	268	NA	NA	[redacted]	23.79	41.64	211.14
Long-term	2021	Prosser	360	202	56.11	149	NA	NA	[redacted]	19.32	33.80	108.85

Long-term	2022	Prosser	119	74	62.18	49	NA	NA		[redacted]	21.41	37.46	120.63
Total and weighted mean	**21**		***11337***	***5063***	***44.7***	***2289***	***363***	***91***		[redacted]	***15.37***	***26.90***	***86.63***

												Treatment benefit relative to Hockersmith	
Strategy	**Year**	**Capture Location**	**# Collected**	**#** ***Survived***	**S @ release (%)**	**Released**	**# cons. remature**	**Retained**	**skip remature**	[redacted]	**Transportation Benefit relative to in-river**	**1.66**	**Transportation Benefit relative to Bonneville natural**
Long-term	2011	Lower Granite	111	2	1.80	2	2	-	-	[redacted]	9.80	1.08	3.58
Long-term	2012	Lower Granite	124	10	8.06	10	10	0	-	[redacted]	42.30	4.86	7.49
Long-term	2013	Lower Granite/S.F. Clearwater	134	69	51.49	69	69	0	-	[redacted]	144.18	31.02	47.01
Long-term	2014	Lower Granite/Fish Cr.	122	37	30.33	35	35	2	2	[redacted]	71.24	18.27	52.41
Long-term	2015	Lower Granite/S.F. Clearwater/Fish Cr.	140	43	30.71	24	22	21	18	[redacted]	127.53	18.50	59.58
Long-term	2016	Lower Granite/S.F. Clearwater	227	120	52.86	37	19	101	77	[redacted]	243.30	31.85	851.10
Long-term	2017	Lower Granite	269	59	21.93	98	21	58	29	[redacted]	91.07	13.21	42.55
Long-term	2018	Lower Granite	259	177	68.34	79	50	99	1	[redacted]	197.73	41.17	102.05
Long-term	2019	Lower Granite	288	121	42.01	40	39	85	58	[redacted]	174.45	25.31	114.84
Long-term	2020	Lower Granite/Little Goose	137	71	51.82	66	8	63	61	[redacted]	215.19	31.22	100.53
Long-term	2021	Lower Granite/Little Goose	56	22	39.29	65	4	18	11	[redacted]	163.13	23.67	76.21
Long-term	2022	Lower Granite/Little Goose	183	79	43.17	22	11	57	TBD 2023	[redacted]	179.25	26.01	83.74
Total and weighted mean	**10**		***2050***	***810***	***39.5***	***547***	***290***	***504***	***257***	[redacted]	***164.07***	***23.59***	***76.65***
Long-term	2020	Little Goose	49	18	36.7	1	1	17	3	[redacted]			
Long-term	2021	Little Goose	12	1	8.3	0	0	1	1	[redacted]			
Long-term	2022	Little Goose	46	10	21.7	0	0	10	TBD 2023	[redacted]			
Total and weighted mean			***107***	***29***	**27.10**	***1***	***1***	***28***	***4***	[redacted]			
Long-term	2022	Lower Monument	***25***	***3***	**12.00**	***1***	***1***	***2***	TBD 2023	[redacted]			
												201.00	***90.00***
												119	***83.00***

Strategy	**Year**	**Location**	**# Collected**	[redacted]	**# @ Bonneville**	**Return Rate to Bonneville (%)**

Natural repeat	2004	Bonneville Dam	1125	[redacted]	4	0.36
Natural repeat	2005	Bonneville Dam	572	[redacted]	1	0.17
Natural repeat	2006	Bonneville Dam	1452	[redacted]	9	0.62
Natural repeat	2007	Bonneville Dam	1967	[redacted]	12	0.61
Natural repeat	2008	Bonneville Dam	2630	[redacted]	18	0.68
Natural repeat	2009	Bonneville Dam	2454	[redacted]	11	0.45
Natural repeat	2010	Bonneville Dam	1740	[redacted]	6	0.34
Natural repeat	2011	Bonneville Dam	1391	[redacted]	7	0.50
Natural repeat	2012	Bonneville Dam	1486	[redacted]	16	1.08
Natural repeat	2013	Bonneville Dam	1278	[redacted]	14	1.10
Natural repeat	2014	Bonneville Dam	1728	[redacted]	10	0.58
Natural repeat	2015	Bonneville Dam	904	[redacted]	0	0.00
Natural repeat	2016	Bonneville Dam	1610	[redacted]	1	0.06
Natural repeat	2017	Bonneville Dam	837	[redacted]	0	0.00
Natural repeat	2018	Bonneville Dam	896	[redacted]	6	0.67
Natural repeat	2019	Bonneville Dam	820	[redacted]	3	0.37
Natural repeat	2020	Bonneville Dam	1222	[redacted]	4	0.33
Natural repeat	2021	Bonneville Dam	-	[redacted]	-	-
Natural repeat	2022	Bonneville Dam	-	[redacted]	-	-
Total	18		24112	[redacted]	122	***0.52***

B: Publications

Kelt steelhead have been largely understudied. Our initial interest came from the availability of kelt steelhead at the Chandler Juvenile Collection Facility on the Yakima River coupled with Atlantic salmon kelt reconditioning literature (Gray et al. 1987; Crim et al. 1992; Johnston et al. 1992). Below is a brief chronological bibliography of kelt steelhead-related literature with contributions associated with this project noted with an *Indicates articles directly associated with BPA project 2007-401-00.
#Indicates articles where material contributions were made from BPA project 2007-401-00.

Everman (1895) surveyed fish populations in the Snake River and noted local knowledge that steelhead don't die after spawning and must return to the sea.

Whitt (1954) estimated that approximately 2% of adult Clearwater River steelhead were repeat spawners. Unfortunately, Whitt's estimates were conducted after the construction of two hydropower facilities on main-stem river sites; the estimates also relied on scale analysis, which may have resulted in an underestimation of kelt abundance due to scale reabsorption.

Busby et al. (1996) reported iteroparity rates in excess of 17% for winter run steelhead in the Kalama River (lower Columbia River).

*Evans (2003) estimated that 17% of the Snake River steelhead spawning population was observed as kelts in the Lower Granite Dam juvenile by-pass facility during a 10-week monitoring period in 2000.

Wertheimer and Evans (2005) noted high kelt abundance and poor emigration survival of steelhead from upper Columbia River tributaries to the ocean as the underlying limiting factor inhibiting iteroparity.

Evans et al. (2008) identified transportation of downstream-migrating kelts around hydropower dams as a potential method to improve kelt survival.

Keefer et al. (2008) sampled steelhead kelts at main-stem hydropower dams from 2001 to 2004 and estimated that between 0.5% and 1.2% of those fish from the Snake River and tributaries and 2.9% to 9.0% of fish from the Lower Columbia exhibited an iteroparous life history.

*Narum et al. (2008) used microsatellite genotypes to identify kelt individuals to upstream populations of origin. Repeat spawning was negatively correlated with body size and iteroparity may be a valuable source of genetic variability and a conservation priority, especially in years with poor recruitment.

Scott and Gill (2008) anthropogenic modifications to the environment may select against repeat spawning.

Seamons and Quinn (2010) reported that repeat spawning adults have life-time reproductive success more than twice that of one-time spawners, and the average number of offspring produced by both male and female repeat spawners is much higher (1.9-time higher females and 2.7 times higher for males).

Nielsen et al. (2011) analyzed molecular genetic data across various life-history types (single versus repeat-spawning groups) of steelhead inhabiting the Ninilchik River in Alaska. They concluded that steelhead inhabiting this river formed a single panmictic spawning population, suggesting that iteroparity is a ubiquitous trait, potentially governed solely by environmental conditions that are more or less conducive to the survival of post-spawned adults. This is consistent with the fact that expression of iteroparity persists among interior Columbia River steelhead despite decades of passage-related selection against this life history type. Nonetheless, we are not aware of any study that has quantitatively established the presence or absence of specific genetic factors that contribute to iteroparity. Given the lack of conclusive evidence either supporting or negating a genetic component for iteroparity it is reasonable to conclude that enhancing the potential for iteroparity is a risk-averse strategy for steelhead.

*Caldwell et al. (2013) found that a feed restricted regime arrested reproductive development and affected factors associated with energy balance purported to play a role in initiating reproductive development within 2-4 months after spawning.

#Courter et al. (2013) found evidence for variable maternal resident contribution rates to anadromous returns based on otolith microchemistry relative to water chemistry in the Yakima River.

*Hatch et al. 2013 established the sex ratio of kelts being skewed toward female 92% over a collection 9,738 fish. Further reporting described the reconditioning process, survival rates, condition of fish, and release strategies.

Null et al. (2013) reconditioned post-spawned hatchery-origin steelhead for a short period of time at Coleman Hatchery on the Sacramento River and achieved return rates of 26%. Radio tracking showed that some of the reconditioned kelts did not migrate to the estuary and instead resided in the lower Sacramento River until the next spawning run.

*Penney and Moffitt (2013) findings indicate that the physiological processes causing rapid senescence and death in semelparous salmon are not evident in steelhead and recovery begins in freshwater.

*Caldwell et al. (2014) found that ration restriction after spawning arrested ovarian development by 10 weeks after spawning.

*Buelow and Moffitt (2014) found kelts in good external condition had plasma profiles similar to downstream-migrating smolts. In addition, they found more than 80% of kelts ranked in good external condition had smolt-like body silvering. Migrating kelts had Na+, K+ ATPase activity that was significantly elevated over hatchery fish at the time of spawning.

#Hernandez, K., Copeland, T., Wright, K. 2014. Quantitative Assessment of Scale Resorption in Migrating and Spawning Steelhead of the Snake River Basin. Transactions of the American Fisheries Society 143:1562-1568.

*Penney and Moffitt (2014a) found no evidence of significant interannual variation in lipid, protein, and energy density of sexually mature steelhead and steelhead kelts. Postspawning survival of steelhead is likely limited by the low energy density of kelts.

*Penney and Moffitt (2014b) investigated energy change in kelt steelhead and found liver fatty acids were depleted more rapidly than muscle.

Moore et al. (2014) iteroparity provides extra population resilience for anadromous salmonids. “Life history strategies can buffer individuals and populations from environmental variability. For instance, it is possible that asynchronous dynamics among different life histories can stabilize populations through portfolio effects.”

Keefer et al. (2016) assessed sea-to-sea survival of 1,212 adult steelhead from the Snake and Columbia rivers. They found survival from Snake River tributary entry to postspawn kelt was 14% to 17% and kelt outmigration survival through the lower Snake River dams was 31% to 39% and 13% to 20% through all eight main-stem dams.

*Matala et al. (2016) identified kelt steelhead collected at Lower Granite Dam to A-run (82%) and B-run (18%) components.

*Penney, Z.L., C.M. Moffitt, B. Jones, B. Marston. 2016. Physiological comparisons of steelhead kelts emigrating from the Situk River, AK and Clearwater River, ID. Environmental Fish Biology Vol. 99 No. 4.

*Trammell et al. (2016) compared kelt reconditioning management strategies and found that long-term reconditioning resulted in approximately 5 times more repeat spawners than the next best strategy or control group.

*Bosch et al. (2017) provided an outreach article to The Osprey describing the benefits of steelhead kelt reconditioning to wild steelhead populations.

Copeland et al. (2017) reports on the life history diversity across management areas in the Snake River and finds broad overlap among populations in several areas forming a gradient in life history characteristics rather than a dichotomous break.

*Pierce, A.L., J.W. Blodgett, T.D. Cavileer, L.R. Medeiros, J. Boyce, L.K. Caldwell, W.J. Bosch, R. Branstetter, D.E. Fast, D.R. Hatch, and J.J. Nagler. 2017. Reproductive development in captive reconditioned female steelhead kelts: evidence for consecutive and skip spawning life histories. Canadian Journal of Fisheries and Aquatic Sciences 74(7): 1049-1060.

*Jenkins LE, Pierce AL, Graham N, Branstetter R, Hatch DR, and Nagler JJ. 2018. Reproductive performance and energy balance in consecutive and skip repeat spawning reconditioned female steelhead trout Oncorhynchus mykiss. Transactions of the American Fisheries Society.

Copeland et al. (2019) investigated patterns of iteroparity in wild Snake River steelhead and reported a greater percentage of skip spawners relative to consecutive spawners. Additionally, survival to a second spawning was greater for smaller fish.

*Jenkins LE, Pierce AL, Graham ND, Medeiros LR, Hatch DR, and Nagler JJ. 2019. Elevated plasma triglycerides and growth are early indicators of reproductive status in post-spawning female steelhead trout Oncorhynchus mykiss. Conservation Physiology.

*Jenkins et al. (2020) found no support for the idea of a trade-off between current and future reproduction instead reproductive effort and its allocation to egg size and fecundity depended on energetic status during early oogenesis.

*Medeiros et al. (2020) developed a cost-comparable, non-radioisotope method for quantifying salmonid plasma growth hormone and IGF-I using commercially available reagents.

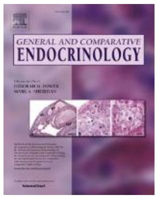
*Jenkins et al. (2022) that energy balance at spawning and feeding after spawning regulated reproductive schedules. These results show that the critical period model of salmonid maturation applies to regulation of repeat spawning, and that the reproductive decision window extends into the first 10 weeks after spawning. Document is on next page.

General and Comparative Endocrinology 332 (2023) 114181

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen



Feeding after spawning and energy balance at spawning are associated with repeat spawning interval in steelhead trout

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ARTICLE INFO

Keywords:
Growth hormone
Insulin-like growth factor-I
Energy balance Reproductive decisions Iteroparity
Recovery from spawning

ABSTRACT

Consecutive and skip repeat spawning (1- or ≥2-year spawning interval) life histories commonly occur in seasonally breeding iteroparous fishes. Spawning interval variation is driven by energetic status and impacts fisheries management. In salmonids, energetic status (either absolute level of energy reserves or the rate of change of energy reserves, i.e., energy balance) is thought to determine reproductive trajectory during a critical period ~1 year prior to initial spawning. However, information on repeat spawners is lacking. To examine the timing and the aspects of energetic status that regulate repeat spawning interval, female steelhead trout (*Oncorhynchus mykiss*) were fasted for 10 weeks after spawning and then fed *ad libitum* and compared to *ad libitum* fed controls. Plasma growth hormone (GH) and insulin-like growth factor-I (IGF-I) levels were measured to assess long-term energy balance. Plasma estradiol levels showed that some fish in both groups initiated a consecutive spawning cycle. In fasted fish, GH was lower at spawning in consecutive versus skip spawners. In consecutive spawners, GH was higher at spawning in fed versus fasted fish. These results suggest that fish with a less negative energy balance at spawning initiated reproductive development in the absence of feeding, but that feeding during the post-spawning period enabled initiation of reproduction in some fish with a more negative energy balance at spawning. Thus, both energy balance at spawning and feeding after spawning regulated reproductive schedules. These results show that the critical period model of salmonid maturation applies to regulation of repeat spawning, and that the reproductive decision window extends into the first 10 weeks after spawning.

1. Introduction

Skip spawning (≥2-years spawning interval) commonly occurs in annually breeding iteroparous teleosts and significantly impacts the management of fish stocks of conservation and economic importance (McBride et al., 2015; Rideout et al., 2005; Rideout and Tomkiewicz, 2011). Skip spawning is attributed to the impacts of the environment on the condition of the fish, such that individuals that experience low quality environments (nutritional or otherwise) defer spawning to increase the chance of successfully reproducing in the future (McBride et al., 2015; Rideout et al., 2005; Rideout and Tomkiewicz, 2011). However, the physiology underlying variation in spawning interval is not well understood.

Anadromous steelhead trout (*Oncorhynchus mykiss*, steelhead) display both consecutive (1-year spawning interval) and skip spawning life histories (Jenkins et al., 2018; Keefer et al., 2008; Pierce et al., 2017), and provide an experimental model for studying post-spawning life histories in fish. Summer-run steelhead migrate from the ocean into freshwater in late summer and early fall and fast until spawning the following spring (Robards and Quinn, 2002). Repeat spawning, like other expressions of reproductive life history diversity, increases population stability in steelhead populations confronted with environmental variability by creating opportunities for interbreeding with individuals spawned in different years (portfolio effect) (Moore et al., 2014). Kelt

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https://doi.org/10.1016/j.ygcen.2022.114181
Received 29 June 2022; Received in revised form 16 November 2022; Accepted 24 November 2022

reconditioning is a population recovery measure in which post-spawned steelhead (called kelts) are captured, held in freshwater tanks, fed, and released to increase the number of repeat spawners (Hatch et al., 2013). Kelt reconditioning is being implemented for stocks of Columbia River Basin summer-run steelhead listed as threatened under the US Endangered Species Act (Hatch et al., 2013; Jenkins et al., 2018; Trammell et al., 2016). The reproductive potential of both repeat spawning life history types (i.e., consecutive and skip spawners) is greater than that of first time spawners and increases with spawning interval (Jenkins et al., 2018). Summer-run steelhead incur a large energy deficit during their prolonged fasting return migration from the ocean and spawning (Penney and Moffitt, 2014), which likely plays a role in determining repeat spawning schedule. However, the timing of the reproductive decision and the aspects of energetic status that determine repeat spawning schedules in steelhead are not known in detail.

In salmonids, entrance into a reproductive cycle is hypothesized to be determined by energetic status during a seasonally defined critical period (Satterthwaite et al., 2009; Taranger et al., 2010; Thorpe, 1986; 2007), and has mostly been studied in terms of initial maturation (puberty). The critical period during which the physiological decision is made to enter a reproductive cycle is thought to occur approximately one year before spawning (Campbell et al., 2006; Satterthwaite et al., 2009; Thorpe, 2007). Energetic status includes both the absolute level of energy reserves, and the rate of change of energy reserves (energy balance), which are often confounded (Taranger et al., 2010; Thorpe et al., 1998). However, in the original formulation of the critical period model, Thorpe proposed that hormones associated with energy balance provide the permissive signal for initiation of a reproductive cycle (Thorpe, 1986). Initiation requires that an individual exceed a genetically determined threshold for energy reserves or energy balance (Thorpe, 1986; 2007). Individuals that do not initiate a reproductive cycle during the critical period will skip reproduction, deferring for a future annual cycle. This gives rise to a genetically determined, condition-dependent reproductive strategy with alternative tactics (Gross, 1996). Determination of reproductive schedules for repeat spawning is likely similar to that of first-time spawners.

Both energetic status approximately one year before spawning and feeding in the early period following spawning have been shown to impact reproductive schedules in seasonally breeding teleosts. In first- time spawning rainbow trout, feed restriction during the first third of the year prior to spawning resulted in a reduced proportion of fish entering into a reproductive cycle (Bromage et al., 1992). In repeat spawning rainbow trout, restricting feed to a maintenance ration after spawning resulted in reduced plasma estradiol-17β levels within 10 weeks after spawning and no fish maturing as consecutive spawners (Caldwell et al., 2013). This suggests that nutritional conditions during a post-spawning critical period play a role in determining the repeat spawning schedule. In winter flounder (*Pseudopleuronectes americanus*), however, fish in better condition at spawning were more likely to spawn in consecutive years regardless of post-spawning fasting treatments (Burton, 1994), implying that energy reserves at spawning played a larger role in the reproductive decision than feeding during the period following spawning in this species. In repeat spawning hatchery-origin steelhead fed *ad libitum*, growth rate was significantly elevated in consecutive versus skip spawners over the initial 10 weeks after spawning (Jenkins et al., 2019), suggesting that consecutive spawning resulted from greater feeding in that group. However, the percentage of consecutive spawners varies substantially between years and locations in wild reconditioned steelhead kelts, although culture conditions including satiation feeding are constant, suggesting an influence of en- ergy reserves at the time of spawning on the reproductive decision (Hatch et al., 2020; Pierce et al., 2017). Nevertheless, standard fisheries measures of energy reserves such as Fulton's condition factor have not proven to differ between consecutive and skip spawning female steel- head trout at the time of spawning (Jenkins et al., 2019), or to determine entrance into a consecutive reproductive cycle (Jenkins et al., 2020).

Understanding the relative importance of energy reserves and energy balance at spawning and feeding after spawning would provide a clearer picture of how the physiological decision to initiate a reproductive cycle as a consecutive spawner occurs in adult steelhead.

In the present study, we measured hormones in the growth hormone–insulin-like growth factor (GH–IGF) endocrine axis as indicators of long-term energy balance. The GH–IGF axis is the principal physiological system that regulates growth in salmonids, as in other vertebrates, in response to nutrition and the environment (Norbeck et al., 2007; Perez-Sanchez et al., 2018; Sheridan, 2021; Wood et al., 2005). During fasting and under other conditions of negative energy balance, circulating GH levels increase and IGF-I levels decrease as liver IGF-I production be- comes resistant to stimulation by GH (Bergan-Roller and Sheridan, 2018; Bjornsson et al., 2018; Norbeck et al., 2007; Pierce et al., 2011; Pierce et al., 2005a; Pierce et al., 2005b). Decreased IGF-I levels curtail growth, and low plasma IGF-I levels can be used to indicate poor growth status (Beckman, 2011; Perez-Sanchez et al., 2018; Picha et al., 2011; Pierce et al., 2001). The increase in circulating GH has a well-established role in the mobilization of stored lipids (Bergan-Roller and Sheridan, 2018; Norbeck et al., 2007; Sheridan, 1986), and a more speculative role as permissive for protein catabolism (Bjornsson et al., 2018). Thus, elevated plasma GH can be used as an indicator of negative energy balance (Bjornsson et al., 2018; Pierce et al., 2005b). The GH–IGF axis also interacts with the reproductive endocrine axis in fishes at the level of pituitary gonadotrophs, and likely other levels, which has been pro- posed to be a mechanism underlying the effect of energy balance on reproductive decisions (Baker et al., 2000; Campbell et al., 2006; Huang et al., 1998; Luckenbach et al., 2010).

The objective of this study was to describe how different repeat spawning intervals develop in adult female summer steelhead. We aimed to delineate the timing of the repeat spawning decision window and the aspects of energetic status associated with the decision. Based on Thorpe's conceptual model that gonadal development is determined by energy balance during a critical period approximately 1 year before spawning in first time spawners (Thorpe, 1986; Thorpe et al., 1998), and our previous finding that energy balance was more positive in consec- utive spawning steelhead by 10 weeks after spawning (Jenkins et al., 2019), we conducted a fasting experiment to test whether repeat spawn interval was already determined at the time of spawning, or if the de- cision period extends into the time period following spawning, and to describe which aspects of energetic status (energy balance, energy re- serves) were associated with the decision. Post-spawning female steel- head were either fed to satiation for 30 weeks or fasted for the 10 weeks immediately following spawning, and then returned to satiation feeding for the following 20 weeks. The fasting treatment was meant to simulate what occurs in the natural environment, as compared to the access to feed immediately after spawning which typically occurs in captive reconditioning. Reproductive status was retroactively assigned to each fish based on plasma estradiol-17β levels collected at 30 weeks post- spawning when complete separation of fish into two groups was observed. Fulton's condition factor and muscle lipid level were measured to assess energy reserves. Plasma GH and IGF-I levels were measured to assess long-term energy balance starting at the time of spawning, and growth rate was used to assess energy balance in the period following spawning.

2. Methods

2.1 Fish

First-time spawning female steelhead (*Oncorhynchus mykiss*) originating from juveniles raised at Dworshak National Fish Hatchery (DNFH; Ahsahka, ID) were captured upon their return to DNFH after ascending the adult fish ladder on the North Fork Clearwater River in February through April in 2017 and 2018. Fish were held unfed in holding ponds supplied with river water following capture. During

February-April in 2017 and 2018, DNFH staff selected fully mature fish > 74 cm for use as broodstock. Broodstock females in good or fair condition (Hatch et al., 2013), N = 179 in 2017 and N = 120 in 2018, were used for this study and individually marked with passive integrated transponder (PIT) tags (Biomark, Boise, ID) inserted into the pelvic girdle.

2.2 Spawning and sampling

Fish were anesthetized using AQUI-S 20E (AquaTactics Inc., Kirkland, WA; 75 mL $1000L^{-1}$ water) and were manually "air spawned" (Leitritz and Lewis, 1976). Fish were non-lethally sampled for length (fork length, FL, cm), wet body mass (kg), muscle lipid level of the dorsal musculature (ML, %; Fish Fatmeter model 692, Distell Inc., West Lothian, UK), total egg mass, and blood (3 mL). Samples of 25 eggs were retained and weighed for each fish to estimate individual egg mass. These data were used to adjust mass at the time of spawning to account for any residual eggs that were removed both at the 10-week sampling point and at terminal sampling. Non-lethal sampling occurred at spawning and at 10-week intervals thereafter (Jenkins et al., 2019), except for fish from 2017 spawning event 2, which were sampled 11 weeks after spawning before resuming the schedule described. Sampling continued at 10-week intervals until fish were terminally sampled in September, 30 weeks after spawning. At the terminal sampling, data similar to the earlier non-lethal sampling was collected, and then anesthetized fish were euthanized according to Institutional Animal Care and Use Committee guidelines. Fish were dissected, ovary weights recorded, and the number of residual eggs in the body cavity recorded.

2.3 Reconditioning husbandry

Fish were held at DNFH in 4.6 m diameter outdoor tanks, supplied with North Fork Clearwater River water at 200L minute^{-1}, maintained at a water height of 1.5 m, with a seasonally varying temperature profile (4.9 – 11.0°C). Tanks were treated with formalin to control external *Saprolegnia* infestation (Syndel USA, Portland, OR; flow through treatment, 1:6000 for one hour daily). Fish were prophylactically treated for bacterial infection and parasitic gill copepods (*Salmincola californiensis*) via intraperitoneal injection: oxytetracycline (Durvet, Blue Springs, Missouri; 20 mg kg^{-1} body weight) and emamectin benzoate (Sigma-Aldrich, St. Louis, Missouri; 200ug kg^{-1} body weight), respectively, at spawning and at every 10 week sampling thereafter (only as needed for copepods) as previously described (Jenkins et al., 2018).

2.4 Experimental treatment

Fish from each spawning event (N = 3 in 2017, N = 2 in 2018) were randomly divided between two tanks. Due to limitations on the number of tanks available, fish from 2017 spawn event 2 and 3, which were one week apart, were combined into the same tanks. One tank from each spawning event was fed *ad libitum*, and the other tank was fasted. Fish from 2017 spawning events 1 and 3 were fasted for 10 weeks, and fish from 2017 spawning event 2 were fasted for 11 weeks. In 2018, fasting lasted 10 weeks for both spawning events. After fasting, fish were consolidated into one tank per spawning event (2017 spawning events 2 and 3 were combined), and all tanks were fed *ad libitum*. *Ad libitum* feeding consisted of offering a mixture of fish pellets (Biobrood, 6-mm pellet size, BioOregon, Longview, Washington) and boiled krill *Euphausia superba* (Atlantic Pacific Products, Kingston, Rhode Island) top-coated with menhaden oil (Argent Aquaculture, Redmond, Washington) and decapsulated Artemia cysts (American Brine Shrimp, Ogden, Utah) at least 5 times daily until feeding activity stopped (Jenkins et al., 2018).

2.5 Assays

Plasma growth hormone (GH, ng/mL) concentration and plasma insulin-like growth factor-I (IGF-I, ng/mL) concentration were measured by time-resolved fluoroimmunoassay in duplicate technical replicates as previously described (Medeiros et al., 2020). To remove interference from IGF binding proteins, plasma samples were treated by acid–ethanol cryoprecipitation, dried down, and reconstituted before being assayed for IGF-I (Medeiros et al., 2020; Shimizu et al., 2000). Plasma estradiol- 17β (E2, pg/mL) concentration was measured by ELISA (Biosense, Cayman Chemical, Ann Arbor, MI), in triplicate technical replicates after solvent extraction as previously described (Jenkins et al., 2019).

2.6 Morphometric analysis

Mass specific growth rate (MSGR) and Fulton's condition factor (K) were calculated as follows:

$$\mathrm{MSGR}\ (\%\ \text{body mass gain} * \text{day}^{-1}) = 100 * (\text{In}(\text{body mass final}) - \ln(\text{body mass initial})) * \text{days}^{-1}$$

$$K = 100 * \text{body mass(g)} * (\text{fork length(cm)})^{-3}$$

Before calculation of MSGR and K, body mass was adjusted to account for any eggs retained from spawning in the body cavity (Jenkins et al., 2018). Length specific growth rate (LSGR) was calculated the same way as MSGR by replacing body mass with FL.

2.7 Statistical analysis

Our analysis assumed that reproductive status was determined by 10 weeks post-spawning, as shown by our previous work (Jenkins et al., 2020; Jenkins et al., 2019). Our interest was in physiological characteristics that are related to the reproductive decision, which may be established before spawning (e.g., Burton, 1994). Our aim was not to build a predictive model, but instead to describe relationships among the variables measured in the study. Therefore, we used a retrospective analysis to describe differences between groups of fish during the post-spawning period. In addition, we were interested in patterns of response that were similar between years, rather than potential differences between years. Therefore, when year differences in response variables were found, we standardized data to initial values and then evaluated whether years could be combined.

Reproductive status was assigned in early autumn, 30 weeks after spawning, based on complete separation of fish into two E2 concentration groups (high levels = reproductive, low levels = non-reproductive), and necropsy by examining developing ovaries for large (vitellogenic) oocytes. Although reproductive status was assigned 30 weeks after spawning, this does not mean that reproductive status was determined at 30 weeks after spawning. It was likely determined much earlier, possibly before spawning. Female steelhead kelts do not change reproductive status after the 30-week time point (Jenkins et al., 2019). Fed and fasted reproductive and non-reproductive groups were compared at 10-week intervals in a time series starting at spawning. Only fish positively identified by PIT tags through the entire experiment were included in the analysis.

Before further analyses, levels of GH and E2 were log$_{10}$-transformed, and ML was arcsine square root-transformed, to conform to normality assumptions. GH, and IGF-I, and E2 were then standardized to the average for all fish at week 0 (standardized value = x * (average value at week zero)$^{-1}$) in each year to account for differences between years, which would be expected due to yearly variations in environmental and ecological conditions experienced by cohorts of fish before capture. Fasting treatment and reproductive status were combined into a single categorical variable or Group (Fed-Reproductive, Fed-Non-reproductive, Fasted-Reproductive, Fasted-Non-reproductive) to simplify data presentation, assess year effects, and show differences over time. For each response variable, two-way ANOVA was used to assess potential differences between years at each time period, where for each response variable R, R = Group + Year + Group*Year, and where Group is the combined categorical variable. Years were combined when significant year and interaction effects were absent. When significant year or interaction effects were found, effect size and examination of year effects were used to determine whether the effect of year or interaction changed the major result of interest, i.e., effects of Group. Years were combined when the main result was not changed.

Week 0 plasma GH, IGF-I, and E2 levels differed between years before standardization (Table 1). After standardization, year and interaction effects were not found for any response variable at weeks 0 or 20 (Supplemental Table 1). A significant year effect was found for GH at week 10. However, the effect of year was small compared to the effect of group, and there were no significant differences between year in any of the groups at this time period. No other year or interaction effects were detected in the GH data. Therefore, years were pooled for the GH data at each time period. Significant year and/or interaction effects were found for ML at week 10 and E2 at week 30. However, for ML, the effect of year was small compared to the significant group effects found at this time point. For E2, the effect of year was larger than the effect of group, but a difference at this time period was inconsequential for the main results of our study. Therefore, data were pooled for each of these response variables at each time period. A large year effect was found for MSGR at the 20–30-week interval, which was stronger than the effect of group over this interval. This was due to higher MSGR in 2018 versus 2017 over this interval, which was significant in both reproductive groups regardless of fasting treatment. However, no year or interaction effects were detected for MSGR at the 0–10 or 10–20-week intervals. Therefore, years are shown combined for these intervals (Fig. 4) and separately for all intervals (Supplemental Figure 4) for MSGR. Years were combined at each time point for IGF-I, K, and LSGR as no year effects were found.

A mixed-effects model was used to test for time, group, and interaction effects on response variables GH, IGF-I, E2, MSGR, K, LSGR, and ML, where for each response variable R, R = Group + Time + Group*Time, and where group is again the combined categorical variable. Group, Time, and Group*Time were treated as fixed effects, and Subject and Residual as random effects (Supplemental Table 2). The mixed-effects model approach accounts for repeated sampling and missing values, using Restricted Maximum Likelihood (REML) and a Geisser-Greenhouse correction. Where significant Group*Time effects were found, the effect of Time within each group was assessed using a similar mixed effects model, where Time was treated as a fixed effect and Individual and Residual as random effects. For ML, Kruskal Wallis tests followed by Dunn's multiple comparison tests were used to test for changes over time due to non-normality.

Then, where significant Group*Time effects were found, fasting treatment and reproductive status were separated, and two-way ANOVA was used to assess the effects of Treatment and Reproductive status for individual fish at each time point, where R = Treatment + Reproductive Status + Treatment*Reproductive Status (Supplemental Table 3). Where the interaction was significant, ordinary-one-way ANOVA, followed by Tukey's HSD, was employed to test for differences between Groups (combined categorical variable described above).

Finally, changes in log-transformed GH concentration were tested for correlation with change in arc-sin-square root transformed ML, change in K, and MSGR from 0-10, 10–20, and 20–30 weeks, with years combined, using simple linear regression.

Table 1
Mean plasma growth hormone (GH), plasma insulin-like growth hormone-I (IGF-I), and plasma estradiol-17β (E2) values in female steelhead at spawning (week 0). For 2017 and 2018 respectively, N = 81 and 49 (GH), N = 82 and 52 (IGF-I), and N = 79 and 52 (E2).

	GH (ng/mL)	IGF-I (ng/mL)	E2 (pg/mL)
2017	23.53	5.067	841.5
2018	14.06	4.398	1061

All statistical analysis was conducted with PRISM software version 9.4.0 (GraphPad Inc., La Jolla, CA). Results are reported as significant when $P < 0.05$.

2.8 Ethics

Fish care and sampling were conducted in accordance with a protocol reviewed and approved by the University of Idaho Animal Care and Use Committee. All methods were carried out in accordance with ARRIVE guidelines.

3. Results

3.1 Survival and reproductive status

Survival to 30 weeks was 46.4 % (83/179) in 2017 and 43.3 % (52/ 120) in 2018 (Fisher's exact test, $P = 0.6365$, Table 2). Survival below 50 % is expected in this experimental model, with proportions in the present study above average compared to that found in previous studies in steelhead kelts: 38 %, 24 % (Hatch et al., 2013; Jenkins et al., 2019). Survival was similar between years for fed (46.2 % in 2017 and 43.1 % in 2018, Fisher's exact test, $P = 0.7384$) and fasted groups (46.6 % in 2017 and 43.5 % in 2018, Fisher's exact test, $P = 0.7415$), and between treatments in each year (46.2 % fed and 46.6 % fasted in 2017; 43.1 % fed and 43.5 % fasted in 2018, $P > 0.9999$ in each case). Survival did not differ between fed and fasted treatment groups when years were combined (Fisher's exact test, $P > 0.9999$). Of the survivors, 28.9 % (24/83, 2017) and 59.6 % (31/52, 2018) became reproductively active on a consecutive spawning schedule (Fisher's exact test, $P = 0.0006$).

The percentage of repeat spawners that enter a reproductive cycle in consecutive years (the reproductive rate) is expected to differ between years (Hatch et al., 2013; Jenkins et al., 2019; Pierce et al., 2017). Reproductive rate differed between years in fed (33.3 % in 2017 and 68.0 % in 2018, Fisher's exact test, $P = 0.0106$) and fasted groups (24.4

Table 2
Survival to 30 weeks after first spawning and percentage of repeat spawners that survived and entered a reproductive cycle in consecutive years (reproductive rate) in female steelhead experimentally held after first spawning. Shared letters superscripts indicate no significant difference based on Fisher's exact test. Lower-case letters indicate horizontal differences between years within a treatment group, and upper-case letter superscripts indicate vertical differences between treatment groups within a year or with years combined. Note that there are two separate vertical comparisons: survival rate and reproductive rate.

		2017		2018		Total	
Fed	Spawned	91		58		149	
	Survived	42	46.2 %^{aA}	25	43.1 %^{aA}	67	45.0 %^{A}
	Reproductive	14	33.3 %	17	68.0 %	31	46.3 %
	Non-Reproductive	28	66.7 %^{aX}	8	32.0 %^{bX}	36	53.7 %^{X}
Fasted	Spawned	88		62		150	
	Survived	41	46.6 %^{aA}	27	43.5 %^{aA}	68	45.3 %^{A}
	Reproductive	10	24.4 %	14	51.9 %	24	35.3 %
	Non-Reproductive	31	75.6 %^{aX}	13	48.1 %^{bX}	44	64.7 %^{X}
Total	Spawned	179		120		299	
	Survived	83	46.3 %^{a}	52	43.3 %^{a}	135	45.2 %
	Reproductive	24	28.9 %^{b}	31	59.6 %^{a}	55	40.7 %
	Non-Reproductive	59	71.1 %	21	40.4 %	80	59.3 %

Table 3
Correlations between change in log-transformed plasma GH concentration and change in K, change in arc-sin square root transformed ML, and MSGR from 0- 10, 10–20, and 20–30 weeks. Simple linear regression of individual fish values from combined years. Bolded values were significant.

		slope	R-squared	p-value
0–10 weeks	MSGR	**¡ 0.0802**	**0.3568**	**<0.0001**
	K	**¡ 0.0397**	**0.3431**	**<0.0001**
	ML	**¡ 0.0055**	**0.102**	**0.0004**
10–20 weeks	MSGR	**¡ 0.067**	**0.07126**	**0.0036**
	K	**¡ 0.0313**	**0.0575**	**0.0089**
	ML	−0.0083	0.02801	0.0688
20–30 weeks	MSGR	0.01042	0.00242	0.5889
	K	0.01152	0.00939	0.2864
	ML	**¡ 0.0104**	**0.05096**	**0.0121**

% in 2017 and 51.9 % in 2018, Fisher's exact test, P = 0.0368), and was similar between treatments in both years (33.3 % fed and 24.4 % fasted in 2017; 68.0 % fed and 51.9 % fasted in 2018; Fisher's exact test, P = 0.4692 and P = 0.2702 in 2017 and 2018 respectively). Reproductive status did not differ between fed and fasted treatment groups when years were combined (Fisher's exact text, P = 0.2225).

3.2 *Growth hormone*

There was a significant interaction (F (1, 126) = 11.51, P = 0.0009) between reproductive status (P = 0.0001) and fasting treatment (P = 0.0066) on GH at 0 weeks (Fig. 1). Plasma growth hormone (GH) level was significantly lower in the Fasted-Reproductive group than in any other group. From 0 to 10 weeks, GH increased significantly in fasted fish, and decreased in Fed-Reproductive fish. There was a significant interaction (F (1, 121) = 4.874, P = 0.0291) between fasting treatment (P < 0.0001) and reproductive status (P = 0.00032) on GH again at 10 weeks. GH was greater in fasted than in fed fish, and within the fed treatment, GH was greater in non-reproductive than in reproductive fish. GH decreased significantly from week 10 to 20 in all groups and decreased significantly from week 20 to 30 for all groups except for the Fed-Reproductive group. Reproductive status had a significant effect on GH at weeks 20 and 30, (F (1, 125) = 7.185, P = 0.0083; F (1, 124) = 5.864, P = 0.0169, respectively), such that GH was higher in non-reproductive fish. Plasma GH data from 2017 and 2018 are presented separately in Supplemental Figure 1.

Change in log-transformed plasma growth hormone concentration was significantly negatively correlated with change in K and with MSGR at weeks 0–10 and 10–20, and with change in arc-sin square root- transformed ML from 0-10 weeks and 20–30 weeks (P-values < 0.05). R-squared values were higher at early time points after spawning.

3.3 *Insulin-like growth factor*

There were no significant effects of reproductive status, fasting, or their interaction on plasma insulin-like growth factor (IGF-I) level at 0, 20, or 30 weeks after spawning (Fig. 2). There was a significant effect of fasting on IGF-I at week 10 (the end of the fasting period) such that IGF-I was lower in fasted fish (F (1, 128) = 6.044, P = 0.0153). IGF-I increased over time in all groups. At 20 weeks, IGF-I increased in non-reproductive fish over week 0 levels. At 30 weeks, IGF-I increased over week 0 levels in reproductive fish, over week 10 levels in Fed-Non-reproductive fish, and over week 20 levels in Fasted-Non-reproductive fish. Plasma IGF-I data from 2017 and 2018 are presented separately in Supplemental Figure 2.

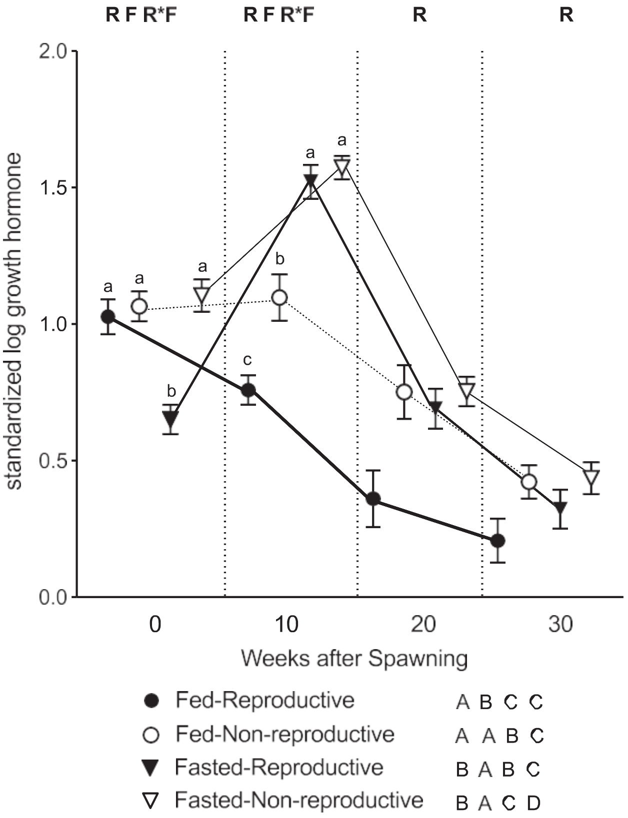

Fig. 1. Plasma growth hormone (GH) in female steelhead sampled at 10-week intervals starting at first spawning. Data from 2017 and 2018 were pooled. Analysis included Fed-Reproductive and Fed-Non-reproductive (closed and open circles, n = 31, 35) and Fasted-Reproductive and Fasted-Non-reproductive (closed and open triangles, n = 24, 44) groups. Symbols indicate mean and bars indicate SEM. Above each sampling point, capital letters indicate significance (R = Reproductive status, F = Fasting Treatment, R*F = Interaction effect). At each sampling point with a significant interaction effect, lower-case letters above the symbols indicate significant differences between groups within the sampling point. Rows of capital letters in the key below the figure indicate significant differences between sampling points over time from left to right (0, 10, 20, and 30 weeks) within the indicated group. Symbols sharing the same letter do not differ significantly.

3.4 *Estradiol-17β*

There was a significant effect of reproductive status on plasma estradiol-17β (E2) level at time 0, such that E2 was higher in reproductive fish (F (1, 127) = 7.411, P = 0.0074) (Fig. 3). In all groups, E2 decreased significantly from 0 weeks to 10 weeks. At 10 weeks, there was a significant effect of the fasting treatment on E2, such that E2 was higher in fed fish (F (1, 128) = 7.205, P = 0.0082). At 20 and 30 weeks, there was a significant effect of reproductive status, such that E2 was higher in reproductive fish (F (1, 125) = 518.3, P < 0.001; F (1, 129) = 1189, P < 0.001, respectively), following significant increases in E2 from 10 to 20 weeks and 20 to 30 weeks. E2 decreased significantly in Fed-Non-reproductive fish from 10 to 20 weeks. E2 in non-reproductive fish increased from 20 to 30 weeks but remained low, only exceeding week 10 levels in Fasted-Non-reproductive fish. Plasma E2 data from 2017 and 2018 are presented separately in Supplemental Figure 3.

3.5 *Growth*

There was a significant interaction (F (1, 129) = 12.81, P = 0.0005) between the fasting treatment (P = 0.0001) and reproductive status (P = 0.0001) on mass specific growth rate (MSGR) during Weeks 0–10 (Fig. 4). MSGR was positive in Fed-Reproductive fish, and negative in Fed-Non-reproductive, Fasted-Reproductive, and Fasted-Non-reproductive fish, indicating weight loss over the week 0–10 time period in the latter three groups. MSGR was higher in Fed-Reproductive fish than all other groups, and lower in Fasted-Non-reproductive fish

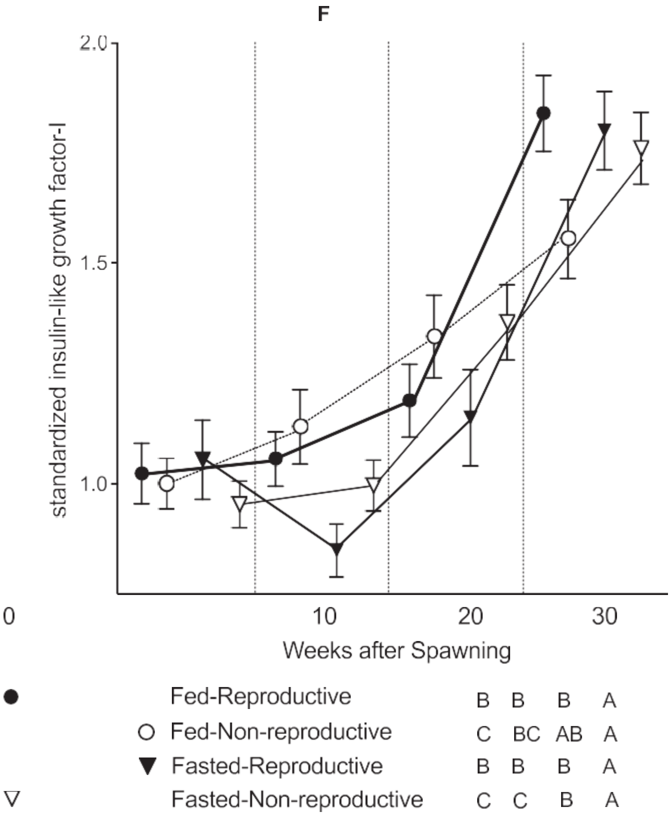

Fig. 2. Plasma insulin-like growth factor (IGF-I) in female steelhead sampled at 10-week intervals starting at first spawning. Analysis is as described in Fig. 1.

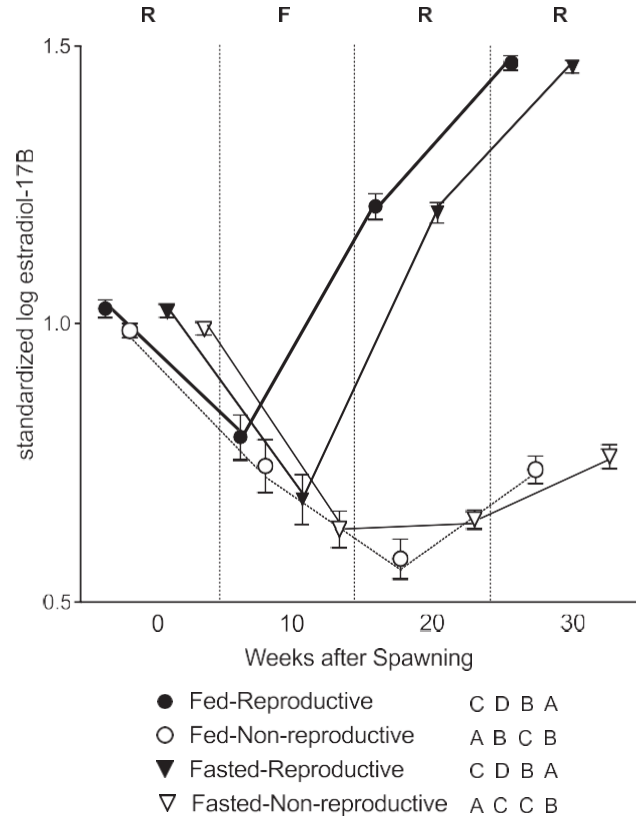

Fig. 3. Plasma estradiol-17β (E2) in female steelhead sampled at 10-week intervals starting at first spawning. Analysis is as described in Fig. 1.

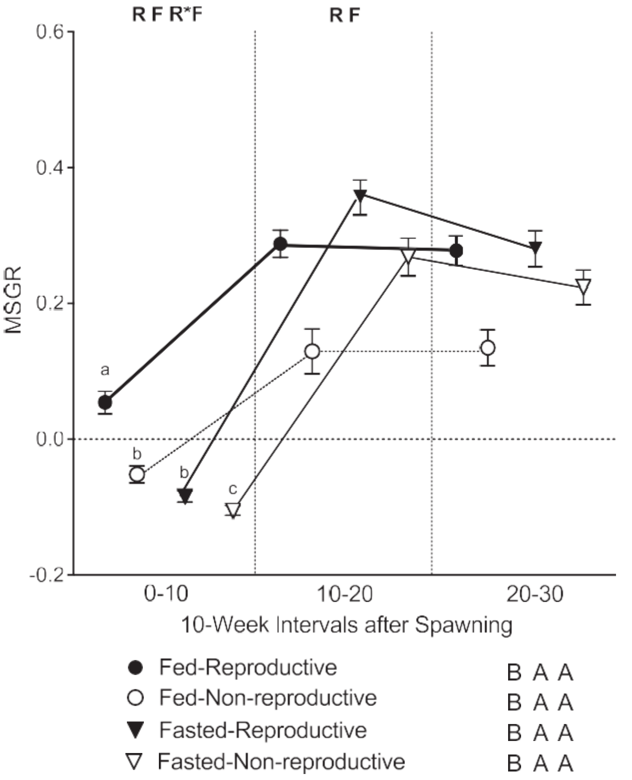

Fig. 4. Mass-specific growth rate (MSGR) in female steelhead sampled at 10- week intervals starting at first spawning. Analysis is as described in Fig. 1. Data from 2017 and 2018 were pooled for combined years analysis for 0–10 weeks and 10–20 weeks only due to significant year effects occuring during 20–30 weeks.

than all other groups. MSGR was negative in Fasted-Reproductive and Fed-Non-reproductive fish but did not differ significantly between these groups. MSGR increased from Weeks 0–10 to Weeks 10–20 for all groups. At Weeks 10–20, reproductive status (F (1, 129) = 18.27, P < 0.0001) and fasting treatment (F (1, 129) = 12.77, P = 0.0005) had significant effects on MSGR. MSGR was higher in reproductive than in non-reproductive fish, and higher in fasted than in fed fish. Because we observed a distinct increase in growth rate at the time when food was made available, we tested and found that growth rate was significantly higher in fasted fish at week 10–20 than in fed fish at 0–10 weeks (P = 0.0001). MSGR decreased in reproductive fish from Weeks 10–20 to Weeks 20–30 in 2017 (Supplemental Figure 4, years analyzed separately). There was a significant effect of reproductive status at weeks 20–30 (F (1, 48) = 8.245, P = 0.0061) in 2018, such that MSGR was greater in reproductive than non-reproductive fish.

There was a significant interaction effect (F (1, 128) = 4.540, P = 0.035) between fasting treatment (P < 0.0001) and reproductive status (P < 0.0001) on length specific growth rate (LSGR) during 0–10 weeks after spawning (1st interval, Fig. 5), such that in fed treatment groups, LSGR was greater in Fed-Reproductive fish than in Fed-Non-reproductive fish and both Fasted-Reproductive and Fasted-Non-reproductive fish. LSGR significantly increased from the 1st to the 2nd (10–20 week) interval for all groups. During the 10–20 weeks after spawning, there was a significant effect of fasting treatment (F (1, 128) = 17.47, P < 0.0001) and reproductive status (F (1, 128) = 11.90, P = 0.0008), but the interaction was not significant (F (1, 128) = 1.657, P = 0.2003). Over this interval, LSGR was significantly higher in fasted than fed fish, and in reproductive than non-reproductive fish. LSGR decreased from the 2nd to the 3rd (20–30 week) interval in Fasted-Reproductive fish. There was a significant effect of the interaction (F (1, 127) = 6.368, P = 0.0129) between reproductive status and fasting treatment

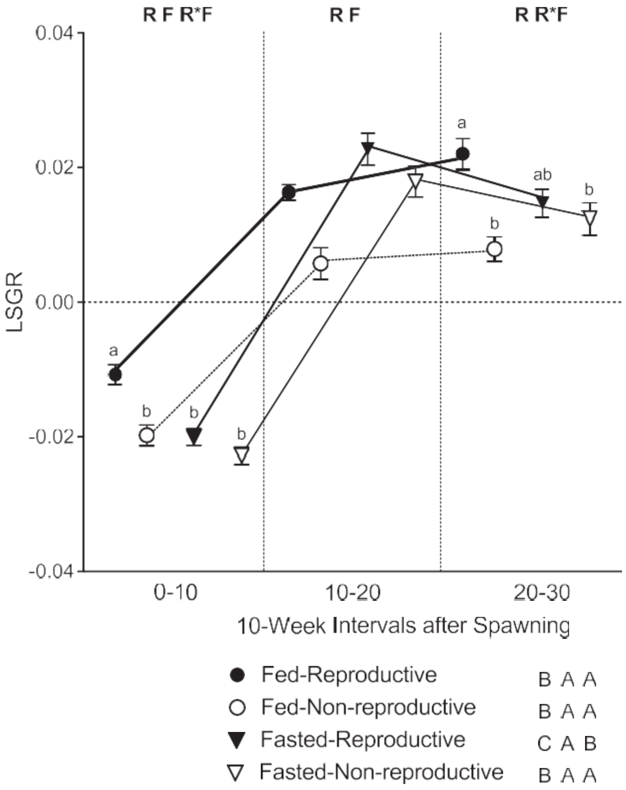

Fig. 5. Length specific growth rate (LSGR) in female steelhead sampled at 10-week intervals starting at first spawning. Analysis is as described in Fig. 1. intervals starting at first spawning. Analysis is as described in Fig. 1.

on LSGR during the 20–30-week interval after spawning. Reproductive status (F (1, 127) = 12.52, P = 0.0006) was also significant during this period. LSGR was significantly higher in Fed-Reproductive than Fed-Non-reproductive fish. Fasted treatment fish were intermediate be- tween these two groups and showed a similar but non-significant pattern. LSGR data from 2017 and 2018 are presented separately in Supplemental Figure 5.

3.6 Condition and lipid levels

At 0 weeks, there was no significant effect of reproductive status, fasting treatment, nor their interaction on Fulton’s condition factor (K, Fig. 6). K increased in Fed-Reproductive fish and decreased in Fasted-Non-reproductive fish from 0 to 10 weeks. At 10 weeks, there was a significant effect of fasting treatment (F (1, 129) = 27.26, P < 0.0001) and reproductive status (F (1, 129) = 11.86, P = 0.0008), but the interaction was not significant (P = 0.0744). K was higher in fed than fasted fish, and higher in reproductive than non-reproductive fish. K increased in all fish from 10 to 20 weeks and again from 20 to 30 weeks. At 20 and 30 weeks, there was a significant effect of the interaction (F (1, 130) = 5.041, P = 0.0264 and F (1, 130) = 6.369, P = 0.0128, respectively) between reproductive status and fasting treatment on K. There was also a significant effect of reproductive status at both 20 and 30 weeks (P < 0.0001, P < 0.0001 respectively). At 20 weeks, Fed- Reproductive fish had higher K than non-reproductive fish and Fasted-Reproductive fish had higher K than Fed-Non-reproductive fish. At 30 Weeks, reproductive fish had higher K than non-reproductive fish. Condition K data from 2017 and 2018 are presented separately in Supplemental Figure 6.

As with K, there were no significant effects of reproductive status, fasting treatment, or their interaction on muscle lipid level (ML, Fig. 7) at 0 weeks. From 0 to 10 weeks, ML decreased in non-reproductive fish. At 10 weeks, fasting treatment (F (1, 128) = 30.24, P < 0.0001) and reproductive status (F (1, 128) = 5.285, P = 0.0231) each had significant

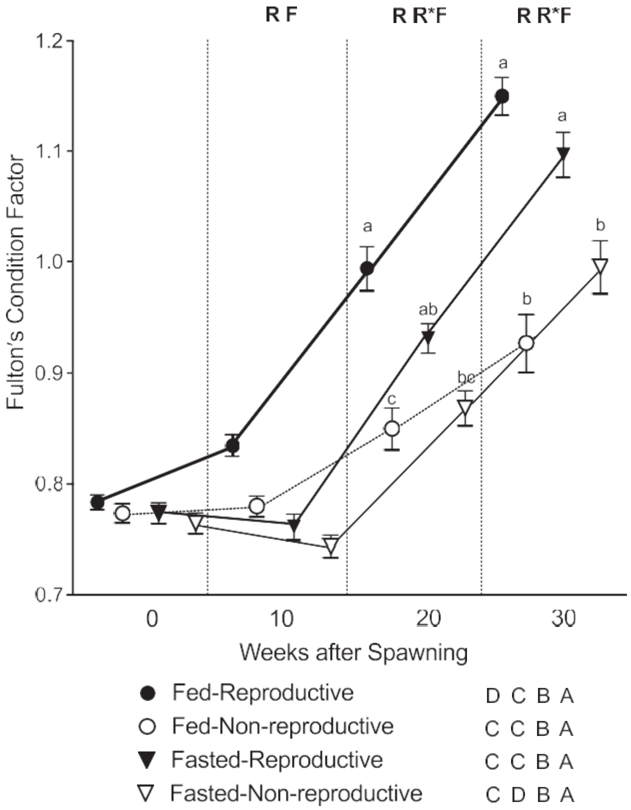

Fig. 6. Fulton’s condition factor (K) in female steelhead sampled at 10-week

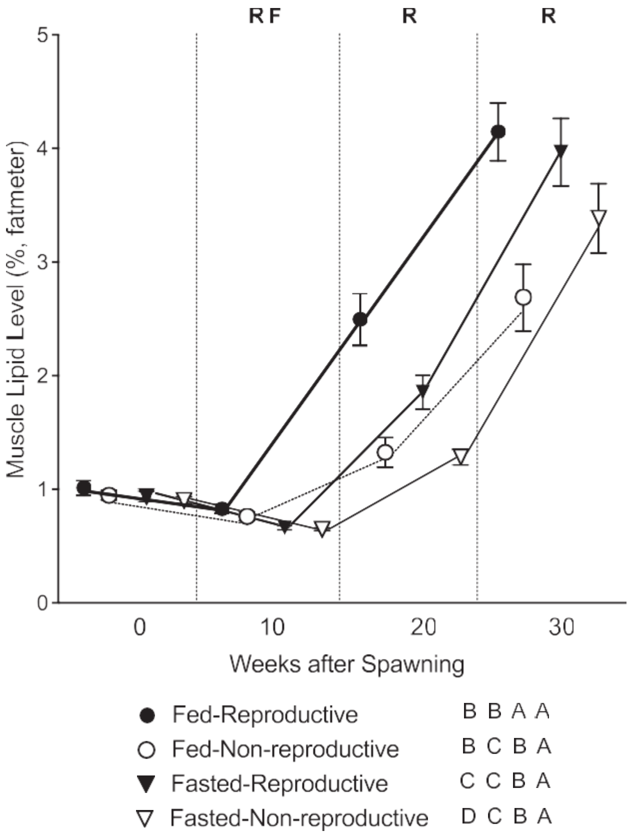

Fig. 7. Muscle lipid level (ML) in female steelhead sampled at 10-week intervals starting at first spawning. Analysis is as described in Fig. 1.

effects on ML, and the interaction was not significant (F (1, 128) = 3.114, P = 0.08). ML was higher in fed than in fasted fish, and higher in reproductive than in non-reproductive fish. ML increased in all groups from 10 to 20 weeks, and in all groups except Fed-Reproductive (P = 0.0764) from 20 to 30 weeks. At 20 and 30 weeks, there was a significant effect of reproductive status (F (1, 129) = 39.01, P < 0.0001, F (1, 129) = 19.60, P < 0.0001) such that ML was higher in reproductive fish. ML data from 2017 and 2018 are presented separately in Supplemental Figure 7.

4. Discussion

Our plasma growth hormone (GH) results suggest that the repeat spawning interval following first spawning in female steelhead (1) is influenced by feeding in the early period following spawning and (2) is associated with energy balance at the time of first spawning. Two aspects of the GH results suggest this. First, within the consecutive spawning fish, GH at the time of spawning was significantly higher in fed than in fasted fish. This was before the fasting treatment was applied, which could only happen if the fasting treatment selected for fish with different GH levels within the consecutive spawning group: the fasting treatment selected fish with lower GH to continue reproductive development, whereas the feeding treatment relaxed that selection, such that some fed fish with higher GH were able to continue with reproductive development. This implies that post-spawning feeding enabled some fish in the fed group with high GH (suggesting a more negative energy balance) to enter into or continue a consecutive reproductive cycle that would not have done so if they had been fasted. Feed restriction in the early period following spawning has been shown to stop a consecutive reproductive cycle (Caldwell et al., 2013). This suggests that energy balance during a decision window that extends into the first 10 weeks after spawning determines repeat spawning interval, extending the critical period model of salmonid maturation to encompass regulation of repeat spawning (Satterthwaite et al., 2009; Thorpe, 2007). Second, within the fasted fish, GH at the time of spawning was significantly lower in reproductive fish (consecutive spawners) than in non- reproductive fish (skip spawners). We did not detect a difference in GH at spawning between consecutive and skip spawning fed fish. We interpret the difference in GH levels in the fasted fish to be due to a difference in energy balance. Lower GH in the fasted consecutive spawning fish suggests that a less negative energy balance at the time of spawning enabled entrance into or continuation of a reproductive cycle. Although we believe that the association of plasma GH level with energy balance likely underlies the pattern in our results, we acknowledge that there are other possibilities, such as a direct interaction between the reproductive endocrine axis and GH.

Further support for the role of energy balance in determining spawning interval is evident in comparisons within each treatment. Fed fish did not differ significantly in GH at the time of spawning despite reproductive status. However, amongst the fed fish at 10 weeks post-spawning, consecutive spawners exhibited both a decrease in GH from spawning levels and lower GH than skip spawners. These changes suggest that consecutive spawners were consuming more food during the post-spawning period. However, surprisingly, a 10-week fast following spawning did not prevent all females from initiating a consecutive spawning cycle. Instead, some fasted females became consecutive spawners, and these displayed lower GH at spawning than those that skipped. Elevated GH levels, as were found in all groups except the fasted consecutive group, suggest that a greater degree of lipid mobilization is being stimulated (Bergan-Roller and Sheridan, 2018; Bjornsson et al., 2018; Sheridan, 1986), consistent with the fish in these groups being in a state of greater negative energy balance. Lower GH levels in the fasted consecutive group suggests a less negative energy balance in those fish, which allowed entrance into or continuation of a spawning cycle despite the challenge of the fasting treatment. GH has the potential to serve as a biomarker of energy balance (positive or negative growth) in some instances (Picha et al., 2011) as it has been shown to respond to both fasting and refeeding (Bjornsson et al., 2018; Gabillard et al., 2006; Norbeck et al., 2007; Pierce et al., 2005b). Although we do not provide direct evidence of differential energy balance at the time of spawning, results from this study suggest that GH may provide a biomarker for energetic status under conditions of extreme energy depletion. Consistent with this interpretation of our GH results, winter flounder *Pseudopleuronectes americanus* with higher condition factor, indicating greater energy reserves, initiated ovarian recrudescence despite feed restriction after spawning (Burton, 1994).

Lower GH in fed consecutive spawning fish at 10 weeks is consistent with the positive growth rates observed in these fish over the first 10 weeks versus the negative growth rates observed in the fed skip spawning fish and the fasted fish, which all had higher GH at 10 weeks than fed consecutive spawning fish. Interestingly, the negative growth in fed skip spawning fish suggests these fish were consuming less than a maintenance ration despite access to satiation feeding. However, GH did not increase from 0 to 10 weeks in this group and was lower at 10 weeks compared to the fasted fish, suggesting that they did resume eating at some point during this period. Together, this suggests intermediate energy balance in fed skip spawners from 0 to 10 weeks between fed consecutive spawners and the fasted fish. Entering a spawning cycle was associated with positive growth rates, elevated plasma triglyceride levels, and increased Fulton's condition factor K over or at the end of the first 10 weeks after spawning in our previous study on female steelhead (Jenkins et al., 2019). This supports the hypothesis that skip spawning can be attributed to poor physiological condition and inadequate nutrition (Rideout et al., 2005; Rideout and Tomkiewicz, 2011), and suggests that physiological assessment involved in the reproductive decision occurs at least in part during the 10 weeks after spawning in the female steelhead model.

GH increased strongly in fasted fish after 10 weeks of fasting, and then decreased over time with refeeding and recovery from spawning. The increase in GH in response to fasting was similar to previous results found in subadult salmonids and other teleosts (Bergan-Roller and Sheridan, 2018; Gabillard et al., 2006; Pierce et al., 2005b), even though these steelhead had undergone an approximately 6-month fasting spawning migration and the physiological stress of spawning before fasting treatments were applied. Similarly, GH increased during a period of increased metabolic demand due to temperature elevation in hybrid striped bass even after an extended period of fasting (Picha et al., 2009). A major established role of GH during fasting is the mobilization of stored lipids (Bergan et al., 2015; Bergan-Roller and Sheridan, 2018; Sheridan, 2021). GH upregulation stimulated lipolysis in coho salmon parr (Sheridan, 1986) and in rainbow trout hepatocytes (Bergan et al., 2013). Steelhead kelts at spawning have very low body lipid reserves (Jenkins et al., 2019; Penney and Moffitt, 2014). The GH increase in the fasted group in the present study indicates an adaptive response to nutritional stress in the post-spawning period and suggests that steelhead are adapted for fasting after spawning, as would be necessary in the natural environment. GH did not increase in fish that had access to feed in the period following spawning, indicating that at least by the end of the 10 weeks these fish were eating and were in a less negative energy balance than the fasted fish, despite the minimal growth that occurred over this time-period. Once all fish were being fed *ad libitum*, GH decreased over time, consistent with the fish entering a less negative energy balance as they recovered from prolonged fasting and spawning. A similar decrease in GH was observed with refeeding in rainbow trout (Gabillard et al., 2006) and hybrid striped bass (Picha et al., 2009). Overall, these results suggest that the GH-IGF axis continued to operate to access stored energy under conditions experienced at spawning and during the following 10 weeks, and consequently that GH is a good indicator of energy balance in spawning steelhead.

The significant negative correlations between change in GH and MSGR and change in K (0–10 weeks, 10–20 weeks) and change in ML (0–10 weeks, 20–30 weeks) further support a relationship between GH status and energy balance. Relationships decreased in strength over time after spawning, suggesting that change in GH serves better as an indicator of energy balance when fish are under conditions of extreme en- ergy depletion, such as after spawning, and decreases in usefulness in steelhead kelts as physiological condition improves.

IGF-I levels were lower in fasted fish after 10 weeks of fasting, and then increased over time with refeeding and recovery from spawning in all fish. Lower IGF-I in fasted fish after 10 weeks is consistent with fasting induced decreases in IGF-I in juvenile salmonids (Gabillard et al., 2006; Kawaguchi et al., 2013; Pierce et al., 2005b). During prolonged fasting, IGF-I decreases to a plateau at a low level, whereas GH continues to increase (Pierce et al., 2005b), which may account for the overall lower responsiveness of IGF-I versus GH in the present study. Despite heightened GH levels during fasting, liver IGF-I production develops resistance to stimulation by GH, redirecting the fasted liver from growth promotion to energy mobilization (Bergan-Roller and Sheridan, 2018; Gray et al., 1992). Although the plasma IGF-I response to the fast was not dramatic, the IGF system is highly complex, with multiple IGF binding proteins (IGFBPs) that modulate the activity of the IGFs and respond to physiological condition, including nutritional stress, plasma cortisol, and reproductive steroids (Norbeck and Sheridan, 2011; Reindl and Sheridan, 2012; Shimizu and Dickhoff, 2017). It is possible that significant regulation of IGF signaling occurs via IGFBPs in the post- spawning state.

During refeeding and recovery from spawning, IGF-I increased over time. The IGF-I increase is consistent with refeeding in rainbow trout (Gabillard et al., 2006; Norbeck et al., 2007) and with a reduction in glucocorticoid-mediated suppression of IGF-I production. Cortisol is chronically elevated during spawning migrations in salmonids (Baker and Vynne, 2014; Carruth et al., 2000; McBride et al., 1986), and has been shown to suppress GH-stimulated increases in plasma IGF-I levels by reducing the liver’s sensitivity to GH (i.e., inducing GH resistance) in a variety of fishes (Kajimura et al., 2003; Madison et al., 2015; Pierce et al., 2005a; Vijayan et al., 2010). As salmonids recover from the physiological stress and nutrient deficiency associated with spawning migrations and reproduction, cortisol returns to basal levels (Medeiros et al., 2016), theoretically allowing plasma IGF-I levels to increase, consistent with what we observed. Increases in IGF-I stimulate anabolic muscle and bone growth in juvenile salmonids (Kawaguchi et al., 2013; Pierce et al., 2001). The increase in IGF-I over the course of the exper- iment would indicate a shift toward anabolic growth, which is supported by the increase in MSGR observed in all groups 10 weeks post-spawning.

Mass specific growth rate was positive in fed consecutive spawning fish, negative in fed skip spawning fish, and negative in all fasted fish during the first 10-week period. Condition factor also increased in fed consecutive spawning fish from 0 to 10 weeks, both observations that have been made previously in fed steelhead kelts (Jenkins et al., 2019). This indicates that fasted fish and fed skip spawning fish were catabo- lizing energy reserves (body lipid and protein stores) to support meta- bolism (Bar, 2014; Navarro and Guti´errez, 1995). Nutrient partitioning during prolonged fasting calls for lipid mobilization until lipids reach a critically low threshold level and can no longer sustain metabolic de- mands, at which time protein catabolism increases dramatically (Bar, 2014; Secor and Carey, 2016). Interestingly, within fasted fish, consec- utive spawning fish lost less weight than skip spawning fish during the fasting period. This suggests that skip spawning fish reached minimum lipid levels and switched from lipid to protein catabolism before or during the time-period between 0 and 10 weeks, which would result in more rapid weight loss in those fish due to the lower energy density of proteins, whereas consecutive spawning fish were still catabolizing lipids. This is consistent with the higher GH level in fasted skip spawning fish at 0 weeks, which would occur when lipid reserves had been depleted to a greater degree, and would be expected to result in greater stimulation of lipid mobilization in those fish (Bergan et al., 2013; 2015; Bergan-Roller and Sheridan, 2018), as was observed (ML decreased from 0 to 10 weeks in fasted skip spawning fish). In summer-run steelhead, lipids in white muscle tissue decreased 94 % from pre-spawn freshwater entry until post-spawning migration, resulting in <1 % muscle lipid, while protein decreased only 18 % over the same period, leaving protein as the remaining energy reserve (Penney and Moffitt, 2014). These results are consistent with the proposal that elevated GH levels are permissive to protein catabolism at low lipid levels (Bjornsson et al., 2018).

We did not detect differences in muscle lipid level (measured with the Fatmeter) or condition factor between consecutive and skip spawning fish in the fasted group at the time of spawning, providing no evidence that the absolute level of energy reserves at the time of spawning determines reproductive status. Consistent with these results, we did not detect significant effects of measures of energy reserves at spawning (Fatmeter reading, condition factor, and plasma triglyceride level) on the consecutive spawning decision in our previous study on fed steelhead kelts (Jenkins et al., 2020). However, Fatmeter reading and condition factor are known to be imprecise measures of energy reserves at low lipid levels (Caldwell et al., 2013; Jenkins et al., 2020; Sutton et al., 2000; Trudel et al., 2005). In contrast, our observations that fasted consecutive spawning fish had lower GH levels at spawning and lost less weight than fasted skip spawning fish during the 10 weeks after spawning provide indirect evidence that lipid reserves at spawning may play a role in the reproductive decision. Overall, our results support the hypothesis that energy balance is more directly involved in the repro- ductive decision versus energy reserves.

Growth rates (MSGR, LSGR) were greater in fasted than fed fish during refeeding at 10–20 weeks, indicating a compensatory growth response (Ali et al., 2003; Sheridan, 2021), similar to what was observed in hybrid striped bass refed after prolonged fasting (Picha et al., 2009). Remarkably, although fed fish had feed available from 0 to 10 weeks after a prolonged fast, migration, and spawning, growth rates were much higher during the 10–20-week refeeding period in the fasted fish versus the 0–10-week refeeding period in the fed fish. Condition factor and muscle lipid levels followed a similar pattern, whereby both increased dramatically from 10 to 20 weeks in fasted fish compared to the smaller changes observed in fed fish from 0 to 10 weeks. These re- sults suggest there is a period of recovery within the first 10 weeks following spawning during which feeding and growth are suppressed. Similarly, rainbow trout fed *ad libitum* following spawning did not in- crease in condition factor or adiposity until 13–16 weeks after spawning (Ahongo et al., 2021). Our results show that recovery can begin to occur without feeding. The physiology underlying this recovery period re- quires further study. One possibility is that recovery from spawning requires a reduction in circulating hormones elevated at spawning that suppress feeding and growth. Cortisol is chronically elevated in female salmonids during their spawning migration (Baker and Vynne, 2014; Carruth et al., 2000; Eliason et al., 2020; Robertson et al., 1961; Westring et al., 2008), increases more during ovulation (Bry, 1985), and eventually decreases after spawning in female rainbow trout (Medeiros et al., 2016). Cortisol is an anorexigenic hormone, suppressing feeding and growth in juvenile rainbow trout (Gregory and Wood, 1999; Mad- ison et al., 2015), channel catfish *Ictalurus punctatus* (Peterson and Small, 2005), and tilapia *Oreochromis mossambicus* (Janzen et al., 2012). The different physiological responses to refeeding observed between the groups of fish could thus be explained by decreases in plasma cortisol and/or other hormones elevated at spawning and during recovery from spawning. Further study is required to investigate this hypothesis.

Growth rates (MSGR, LSGR) were greater in consecutive than skip spawning fish at 10–20 weeks, as were condition factor and muscle lipid level at 20 and 30 weeks, following patterns found in our previous work (Jenkins et al., 2019). Increased feeding and growth are associated with early stages of reproductive development in salmonids (Taranger et al., 2010; Thorpe, 2007; Thorpe et al., 1998).

Estradiol-17β (E2) was slightly higher in consecutive spawning fish at 0 weeks, and higher in fed fish at 10 weeks. This could be due to greater energy reserves leading to increased substrate availability (i.e., cholesterol) for steroid biosynthesis in the consecutive spawning fish and obtained from the feed in fed fish. Although the difference in E2 at spawning was statistically significant, we are unsure that this small elevation in consecutive spawners was biologically significant. Differences between consecutive and skip spawners in E2 at first spawning have not been consistently observed in our previous studies on female steelhead kelts (Jenkins et al., 2019; Pierce et al., 2017). Otherwise, E2 followed the general patterns observed in previous work (Jenkins et al., 2019).

In conclusion, this is the first study we know of to show that both feeding after spawning and energy balance at the time of spawning influence the repeat spawning decision in a female teleost. Our results suggest that the reproductive decision occurs during a critical period during the first 10 weeks following spawning. In addition, our results suggest that the GH–IGF axis operates to access stored energy and regulate growth in the post-spawning state. Future research should continue to utilize this unique model to understand how the growth axis and other endocrine systems interact with environmental factors to influence recovery from spawning and repeat spawning intervals, both to inform population management and to improve species persistence. One strategy for future research into the physiological decision to spawn in consecutive years would be to begin feeding treatments in steelhead prior to spawning. Feeding would be expected to improve energetic status, allowing some fish with poor energetic status to improve prior to the end of the decision window, such that they can become reproductive in consecutive years. Ultimately, understanding reproductive decisions in teleosts requires study of the neuroendocrinology underlying these decisions in the teleost brain (Wootton and Smith, 2015).

CRediT authorship contribution statement

Laura E. Jenkins: Conceptualization, Methodology, Data curation, Investigation, Formal analysis, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Lea R. Medeiros:** Investigation, Writing – review & editing. **Neil D. Graham:** Investigation. **Brian M. Hoffman:** Investigation. **Diana L. Cervantes:** Investigation. **Douglas R. Hatch:** Investigation, Funding acquisition, Resources. **James J. Nagler:** Conceptualization, Methodology, Investigation, Funding acquisition, Resources, Project administration, Supervision, Writing – review & editing. **Andrew L. Pierce:** Formal analysis, Investigation, Conceptualization, Methodology, Software, Validation, Visualization, Writing – original draft, Funding acquisition, Resources, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The Dworshak Kelt Reconditioning Project is the result of collaborative efforts by individuals from a variety of agencies including the Columbia River Inter-Tribal Fish Commission (R. Branstetter, B. Begay, J. Newell, J. FiveCrows, and others), the Nez Perce Tribe Department of Fisheries Resource Management (S. Everett, T. Tall Bull, J. Samuels, J. Sommers, and others), the U.S. Fish and Wildlife Service (A. Feldmann, J. Pike, A. Izbicki, and others), Dworshak National Fish Hatchery, and the University of Idaho (N. Hoffman, T. Cavileer, J. Vrtělová Holbert, and others). We would also like to thank Dr. Chris Williams for statistical consultation. We thank the many managers, biologists, and staff who have maintained the Clearwater River steelhead trout population through the years. This work was funded by the Bonneville Power Administration [Project 2007-401-00] through the Columbia Basin Fish Accords Agreement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2022.114181.

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Presentations 2022

Everett, S. Kelt Reconditioning – Safety Net for Snake River Steelhead. Northwest Fish Culture Concepts, Portland, OR 12/6/22-12/8/22.

Hatch, D.R.Steelhead Kelt Reconditioning and Reproductive Success Evaluation Project. Independent Science Review Panel/Northwest Power and Conservation Council. February 16, 2022.

Hatch, D.R. Steelhead Kelt Reconditioning. Presentation to the Advanced Fisheries Management Class, University of Idaho, February 15, 2022.

Hatch, D.R., R. Branstetter, J. Blodgett, W. Bosch, M. Fiander, A. Pierce, J. Stephenson, S. Everett, N. Graham. Steelhead Kelt Reconditioning and Reproductive Success Studies in the Columbia River. AFS National Meeting, Spokane, WA Aug 21-25 2022. Part of session: Multi-Dimensional Meanings of Fish to the First Peoples of the PNW.

Jenkins* L. E., L. R. Medeiros, N. D. Graham, B. Hoffman, D. L. Cervantes, D. R. Hatch, J. J. Nagler, A. L. Pierce. ENERGETIC STATUS AT SPAWNING AND POST-SPAWNING NUTRITION INTERACT TO INFLUENCE CONSECUTIVE OR SKIP REPEAT SPAWNING IN SUMMER-RUN STEELHEAD TROUT. World Aquaculture Society, San Diego, 2/28/22-3/4/22.

Jenkins, L., L. Medeiros, N. Graham, B. Hoffman, D. Cervantes, Douglas Hatch, James Nagler, Andrew Pierce. Energetic Status at Spawning and Post-Spawning Nutrition Influence Steelhead Repeat Spawning Schedule. AFS National Meeting, Spokane, WA Aug 21-25 2022. Part of session: Repeat Spawning in Steelhead Trout.

Johnson, B. Update on the Kelt facility at Nez Perce Tribal Hatchery. NPCC FW committee webinar, July 6, 2022.

Newell, J., J. Whiteaker, R. Branstetter, D.R. Hatch, J. Stephenson, W. Bosch, J. Blodgett, C. Frederiksen, D. Fast, A. Pierce, L. Medeiros, N. Graham. Repeat Homing of Artificially Reconditioned Steelhead Kelt in the Yakima Basin, WA. AFS National Meeting, Spokane, WA Aug 21-25 2022. Part of session: Repeat Spawning in Steelhead Trout.

Pierce, A., L. Jenkins, L. Caldwell, L. Medeiros, N. Graham, J. Stephenson, R. Branstetter, D.R. Hatch, J. Nagler. Columbia River Basin Steelhead Trout Kelt Reconditioning: Physiology Studies. AFS National Meeting, Spokane, WA Aug 21-25 2022. Part of session: Repeat Spawning in Steelhead Trout.

C: List of Metrics and Indicators

Data Collection Methods

Air Spawning: https://www.monitoringresources.org/Document/Method/Details/5343
Coloration Rating: https://www.monitoringresources.org/Document/Method/Details/5302

Determining Adult Anadromous Salmonid Gender:
https://www.monitoringresources.org/Document/Method/Details/1429

Determining Sex of Adult Steelhead:
https://www.monitoringresources.org/Document/Method/Details/5334

DNA Extraction Kit Protocol:
https://www.monitoringresources.org/Document/Method/Details/1353

Downloading Data from PTAGIS:
https://www.monitoringresources.org/Document/Method/Details/4095

Electrofishing- Determine Electrofisher Settings:
https://www.monitoringresources.org/Document/Method/Details/115

Electrofishing - Fish Processing and Recovery:
https://www.monitoringresources.org/Document/Method/Details/117

Estimating Lipid Content in Muscle Tissue of Adult Salmonids:
https://www.monitoringresources.org/Document/Method/Details/4215

Extracting Fish Plasma to Measure Reproductive Development:
https://www.monitoringresources.org/Document/Method/Details/4239

Fish Wet Weight: https://www.monitoringresources.org/Document/Method/Details/1734

Genetic Sampling and Storage Using Chromatography Filter:
https://www.monitoringresources.org/Document/Method/Details/4087

Identifying Marks/Tags on Fish:
https://www.monitoringresources.org/Document/Method/Details/342

Identifying Steelhead Kelts:
https://www.monitoringresources.org/Document/Method/Details/5310

Measuring Fish Length- Fork length:
https://www.monitoringresources.org/Document/Method/Details/4041

Measuring Fish Length- Mid-orbital hypural length:
https://www.monitoringresources.org/Document/Method/Details/1549

Modified Assessment of Fish Condition:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/22915

Modified Off Ladder Adult Trapping Procedures:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31061

Modified PIT Tag Marking Procedures:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/22818

O. mykiss and O. tshawytscha SNP Marker Sets for PBT and GSI Use in the Columbia River Basin:
https://www.monitoringresources.org/Document/Method/Details/1356

SNP Genotyping on Fluidigm Platform:
https://www.monitoringresources.org/Document/Method/Details/1332

Tissue Sampling for Parentage Based Tagging:
https://www.monitoringresources.org/Document/Method/Details/1432

Tissue Sampling of Salmonids in Nature for Genetic Analysis:
https://www.monitoringresources.org/Document/Method/Details/933

Weights: Green Egg Mass:
https://www.monitoringresources.org/Document/Method/Details/1457

Weights: Sampling for Green Egg Fecundity Estimate:
https://www.monitoringresources.org/Document/Method/Details/1458

Data Analysis/Interpretation Methods

Assessing Genetic Population Structure Using Bayesian Clustering Methods:
https://www.monitoringresources.org/Document/Method/Details/1351

Calculating a Mean, Variance and Standard Deviation:
https://www.monitoringresources.org/Document/Method/Details/4245

Estimating Relative Reproductive Success (RRS):
https://www.monitoringresources.org/Document/Method/Details/696

Estradiol Assay of Fish Plasma Samples:
https://www.monitoringresources.org/Document/Method/Details/5320

Fulton's Fish Condition Factor:
https://www.monitoringresources.org/Document/Method/Details/952

Genetic Assignment Tests Using GeneClass2:
https://www.monitoringresources.org/Document/Method/Details/487

Genetic Stock Mixture Analysis Using the Software Program BAYES:
https://www.monitoringresources.org/Document/Method/Details/488

Modified Analysis of Variance Models:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/22904

Modified Binary Logistic Regression of Multi-Year Monitoring Data:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31060

Modified Calculating the smolt to adult return rate (SAR):
https://www.monitoringresources.org/Document/CustomizedMethod/Details/22932

Modified Growth Rate for Individual Fish:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31057

Modified Integrated Status and Effectiveness Monitoring Program Salmonid Life Cycle Modeling:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31063

Monitor Survival and Mortality Rates of Fish:
https://www.monitoringresources.org/Document/Method/Details/3992

Parentage Analysis Using Cervus:
https://www.monitoringresources.org/Document/Method/Details/1430

Predicting the Accuracy of Genetic Stock Identification:
https://www.monitoringresources.org/Document/Method/Details/1346

Solvent Extraction of Plasma Samples:
https://www.monitoringresources.org/Document/Method/Details/5319

Tests of Significance: T-Test:
https://www.monitoringresources.org/Document/Method/Details/5291

Vitellogenin Assay of Fish Plasma Samples:
https://www.monitoringresources.org/Document/Method/Details/5335

Metrics

Title	Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2
"Stock composition"	Fish	Composition: Fish Species Assemblage (ID: 56)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Natural
"Kelt condition"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult - Outmigrant	NA
"Maturation rate"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult - Returner	NA
"Reconditioned kelt condition"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult Fish	NA
"Kelt homing"	Fish	Distribution of Fish Species (ID: 62)	Fish Life Stage: RANGE: Adult to Adult	NA
"Fecundity"	Fish	Fecundity: Fish (ID: 68)	NA	NA
"Growth rate"	Fish	Growth Rate: Fish (ID: 73)	Fish Life Stage: RANGE: Juvenile to Adult	NA
"Kelt length"	Fish	Length: Fish Species (ID: 75)	Fish Life Stage: Adult - Outmigrant	NA
"Reconditioned kelt length"	Fish	Length: Fish Species (ID: 75)	Fish Life Stage: Adult Fish	NA
"Lipid content"	Fish	Lipid Content (ID: 200)	NA	NA
"Parentage analysis"	Fish	Relative Reproductive Success (RRS) (ID: 88)	Fish Origin: Both	NA
"Reproductive success"	Fish	Reproductive Success (Nb/N) (ID: 89)	Fish Origin: Natural	NA
"Reconditioned kelt survival rate"	Fish	Survival Rate: Fish (ID: 99)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
"Natural kelt survival rate"	Fish	Survival Rate: Fish (ID: 99)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
"Kelt weight"	Fish	Weight: Fish (ID: 206)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both

"Reconditioned kelt weight"	Fish	Weight: Fish (ID: 206)	Fish Life Stage: Adult Fish	Fish Origin: Both

Indicators

Title	Category	Subcategory	Subcategory Focus 1
"Relative reproductive success of artificially reconditioned kelt steelhead"	Fish	Relative Reproductive Success (RRS) (ID: 88)	Fish Origin: Natural